Scheme 1 Solid Phase Protein Synthesis
Native Chemical Ligations in an N- to C- Terminal Direction

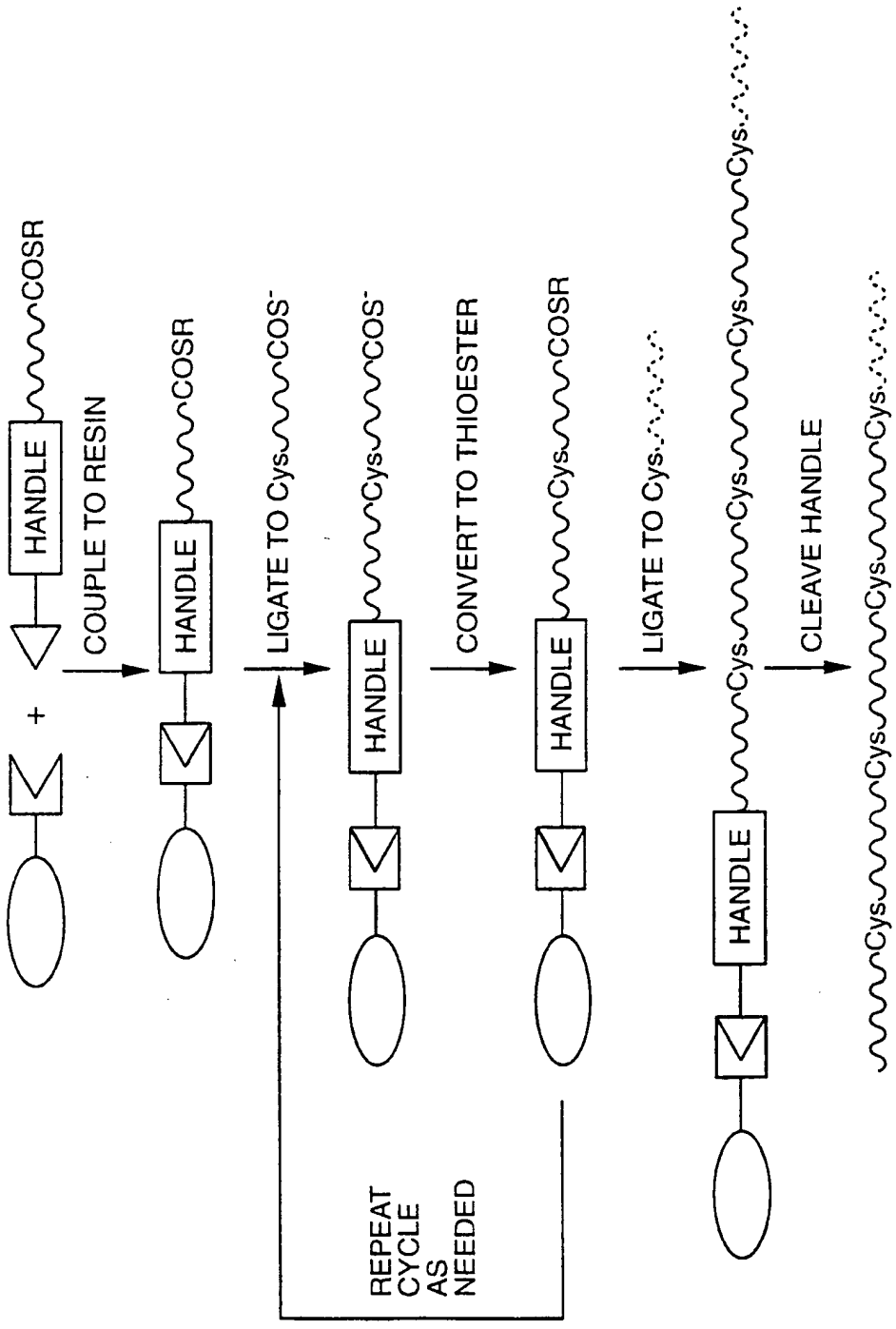


FIG._1

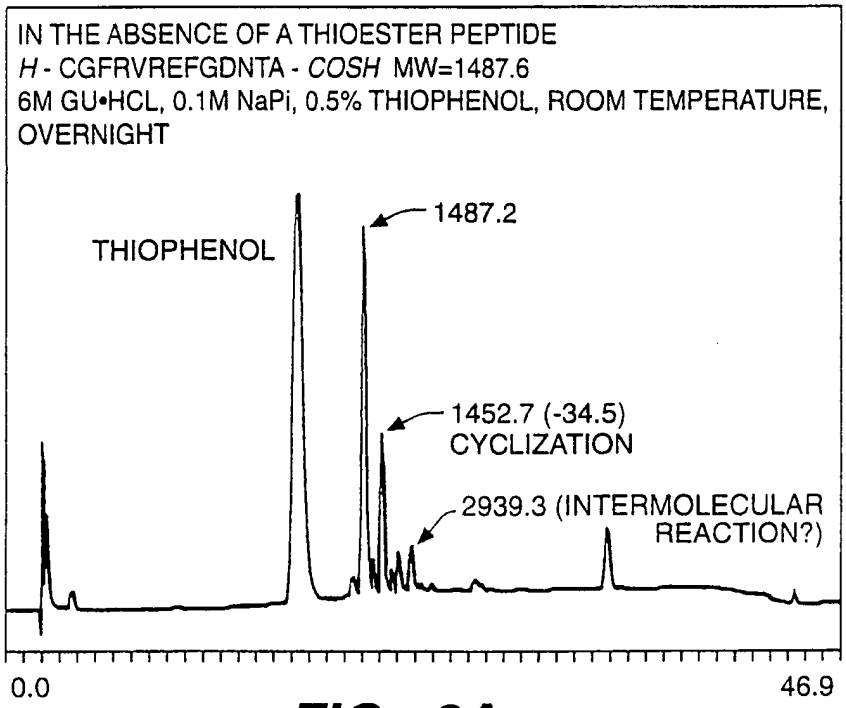


FIG._2A

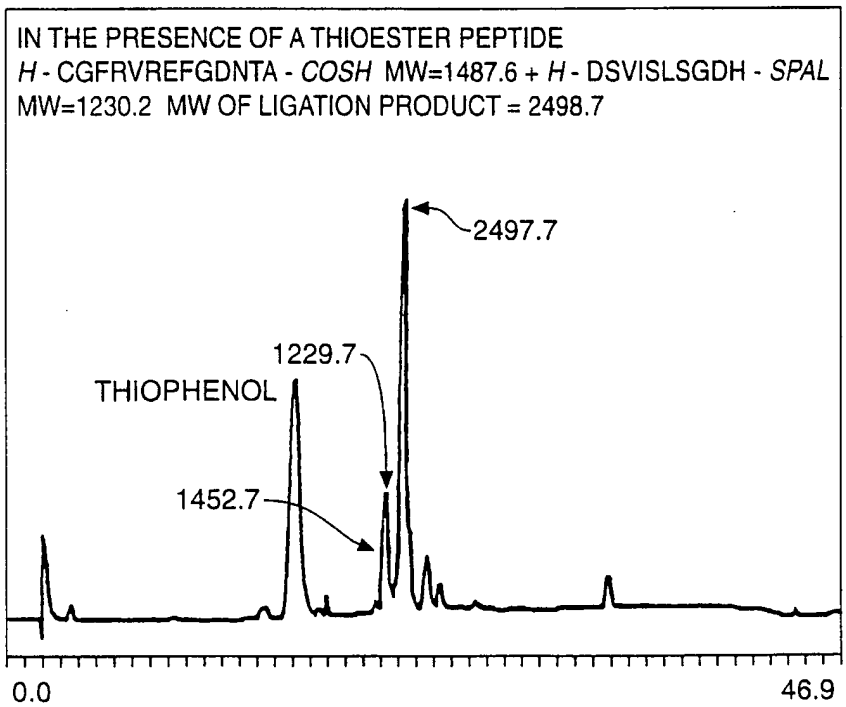


FIG._2B

Cys +COSR Stability Under Ligation Conditions

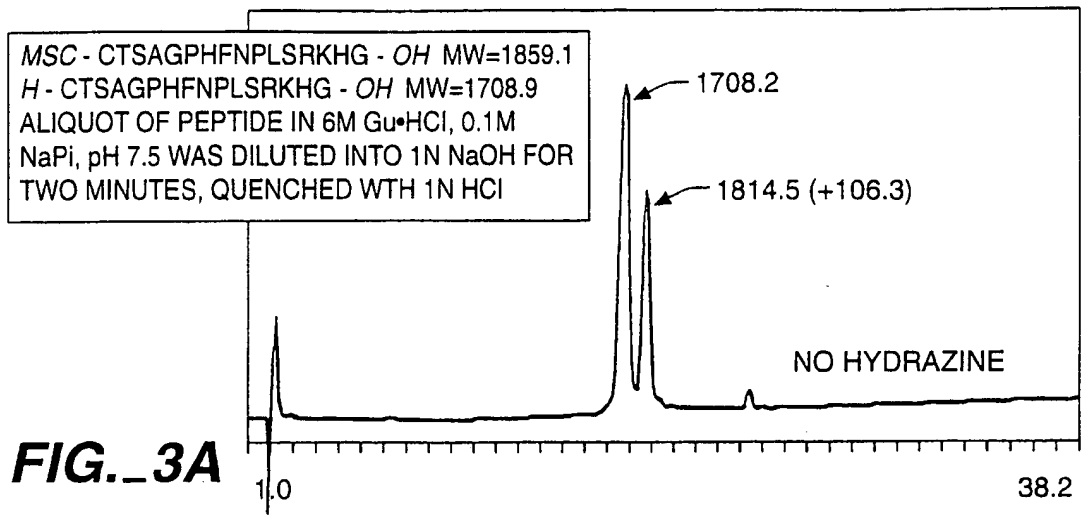


FIG._3A

MSC Removal Experiments

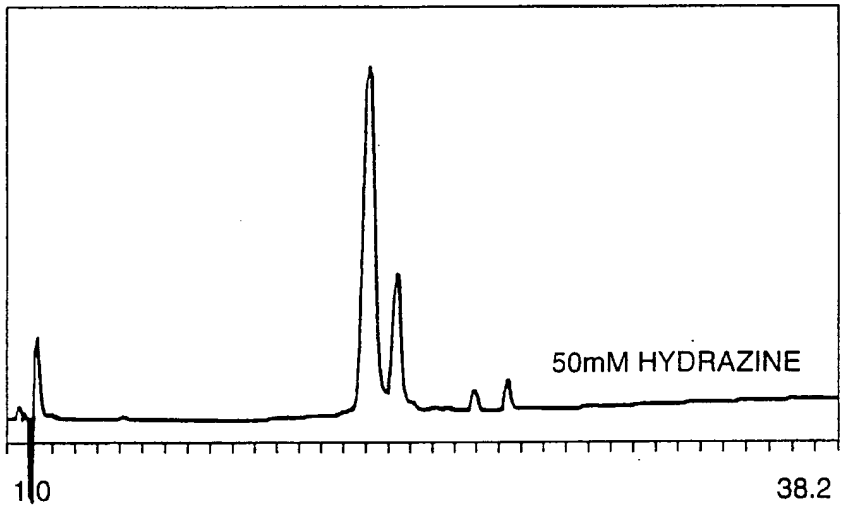


FIG._3B

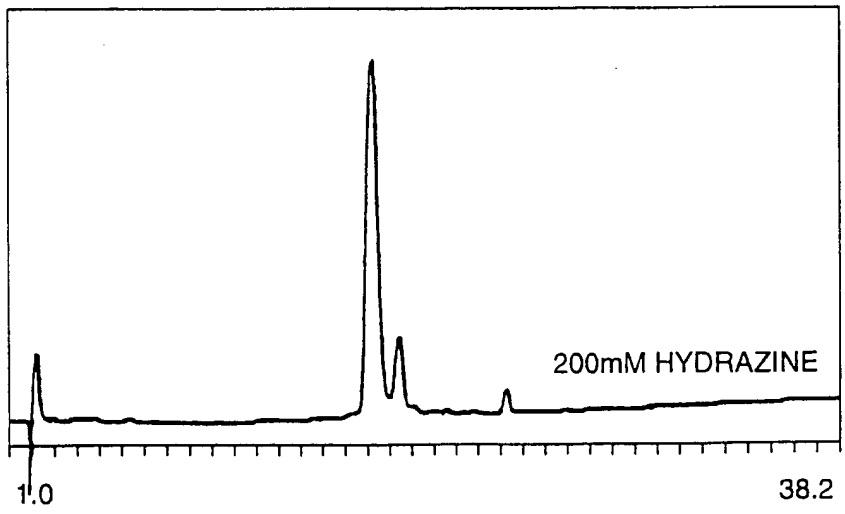


FIG._3C

Lev - MSC - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG - *COSH*
MW=4022.4
H - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG - *COSH*
MW=3745.1
ALIQUOT OF PEPTIDE IN 6M Gu•HCl, 0.1M NaAc, pH 4.6 WAS DILUTED INTO 6M Gu•HCl, 0.1M NaAc, pH 14 FOR TWO MINUTES, QUENCHED WITH 6M Gu•HCl, 0.1M NaAc, pH2.0

MSC Removal Experiments (Cont'd)

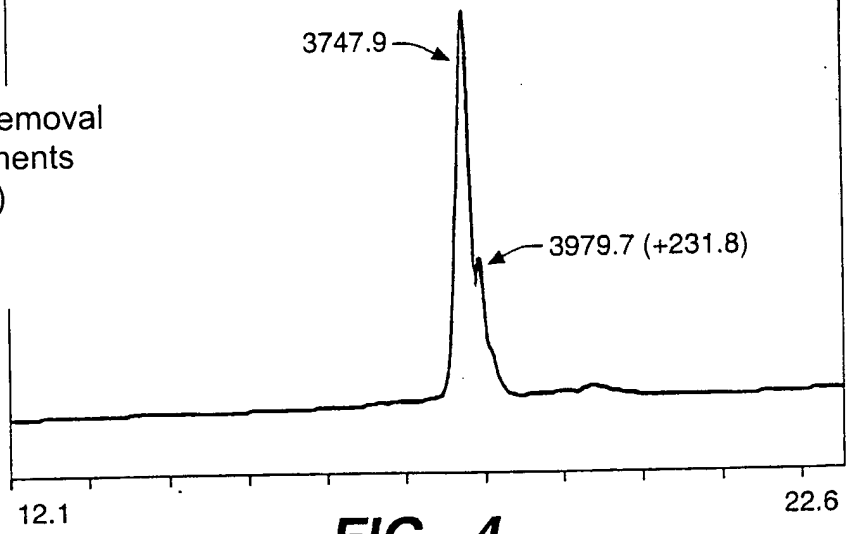


FIG._4

1 21 47
TLQKKIEEIAAKYKHSVVKK**CCYDGACVNNDETCEQRAARISLGPK**CIKAFTECC
VVASQLRANISHKDMQLGR
74

Synthesis of C5a by Solid Phase Chemical Ligations in the N- to C-Terminal Direction

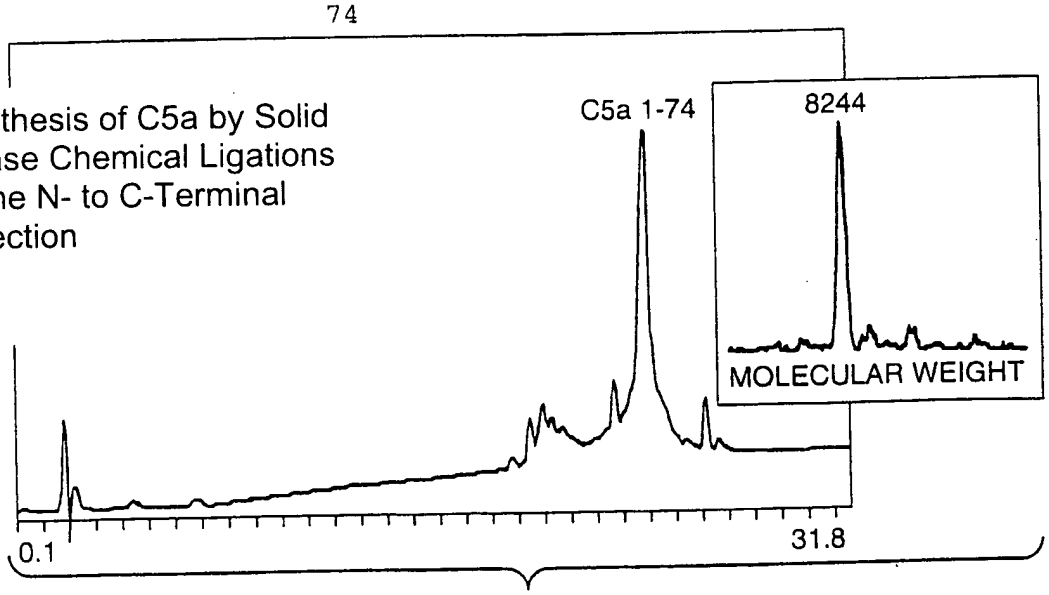


FIG._26

Resin Preparation

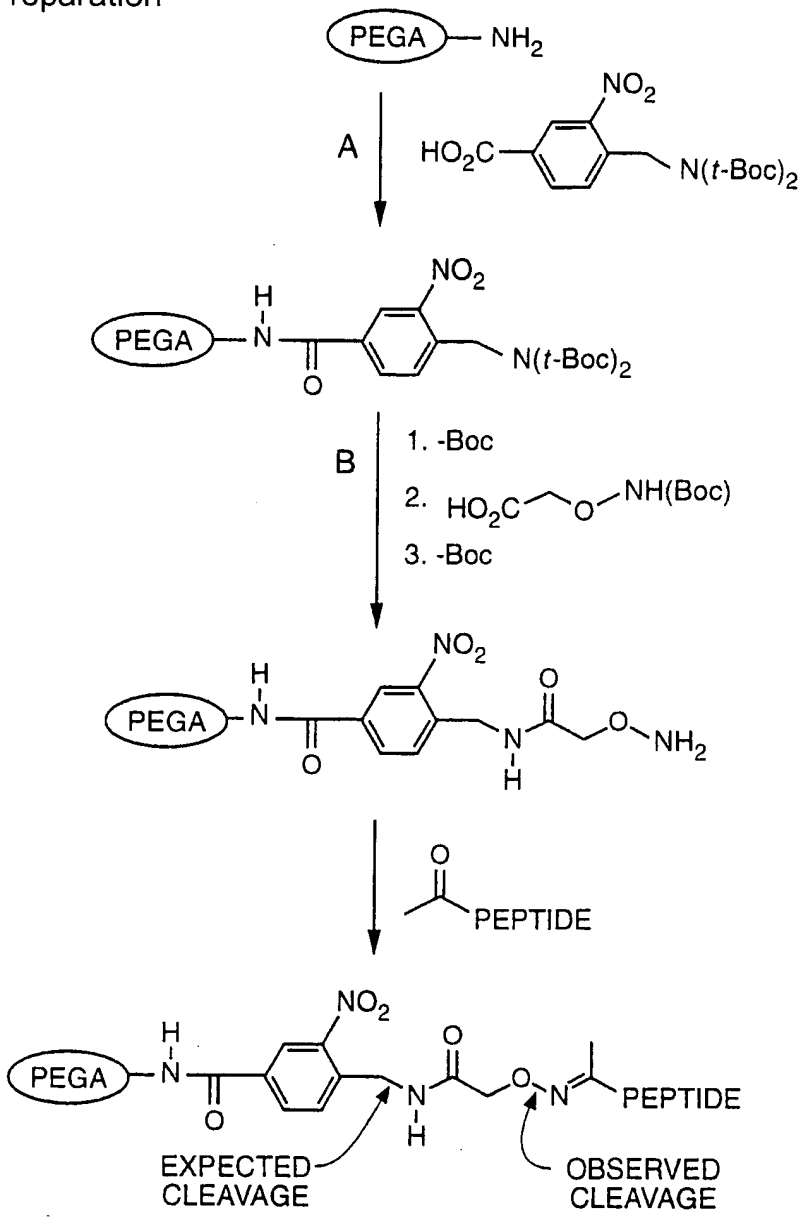
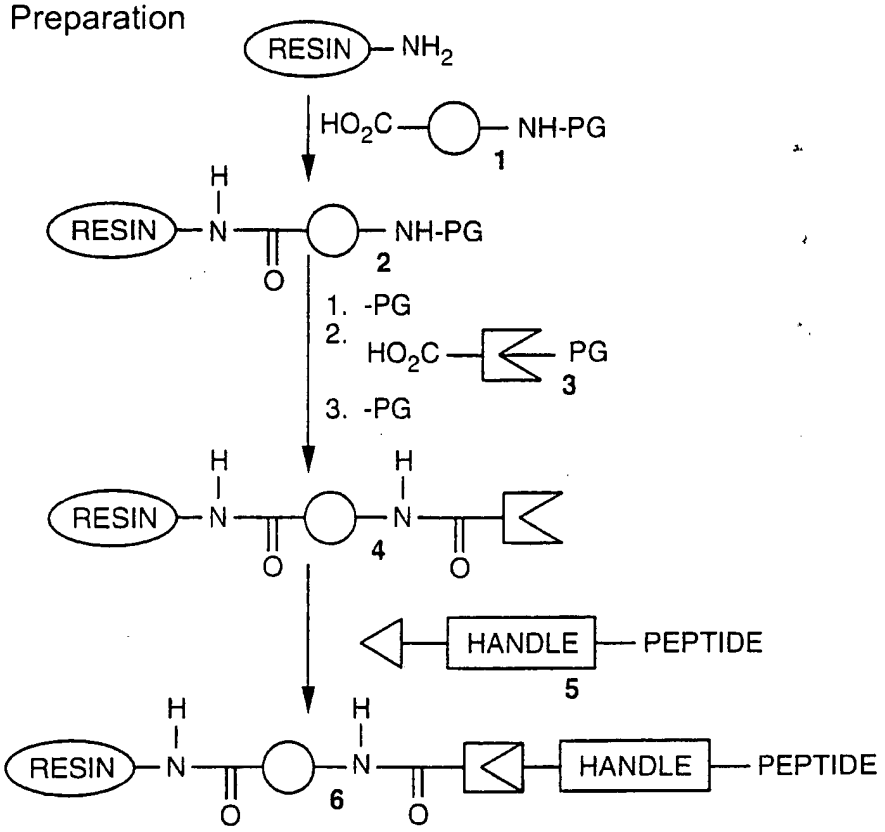


FIG._5A

Resin Preparation



HO_2C — ○ — NH- = CLEAVABLE LINKER USED FOR MONITORING WITH MALDI, ELECTROSPRAY MASS SPECT, ETC...

PG = PROTECTING GROUP

HO_2C — ◁ = FUNCTIONAL GROUP ADDED TO RESIN TO COUPLE WITH PEPTIDE

◁ HANDLE — PEPTIDE = PEPTIDE FUNCTIONALIZED WITH
1. CLEAVABLE HANDLE FOR RELEASE OF PEPTIDE/PROTEIN FROM THE RESIN AT COMPLETION OF SYNTHESIS AND
2. FUNCTIONAL GROUP TO COUPLE TO RESIN

FIG._5B

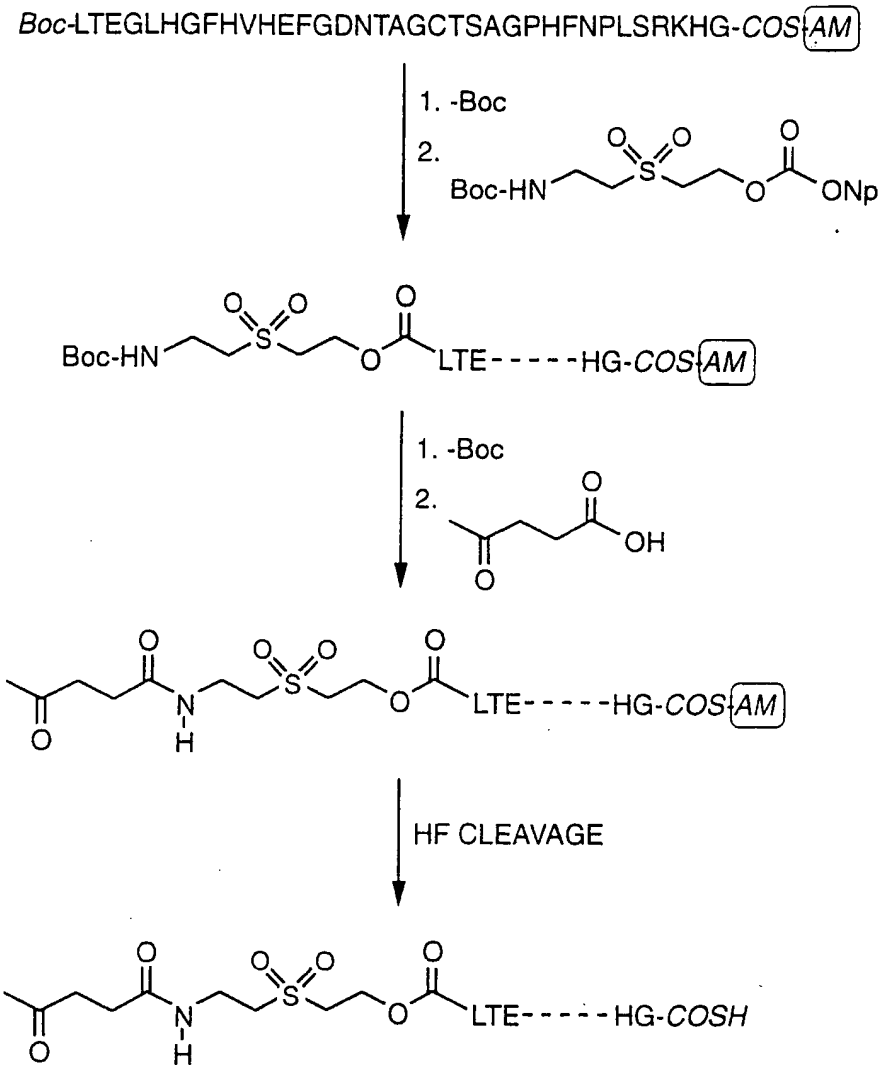


FIG._6

Derivatization of Segment 1
(N-terminal)

Lev - *MSC* - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG - *COSH* (1)
+ Resin - PCL - ONH2

↓ 1. pH 4.6, .6M Gu•HCl, 0.1 ACETATE

Resin - *PCL* - *oxime* - *MSC* - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG - *COSH* (1)

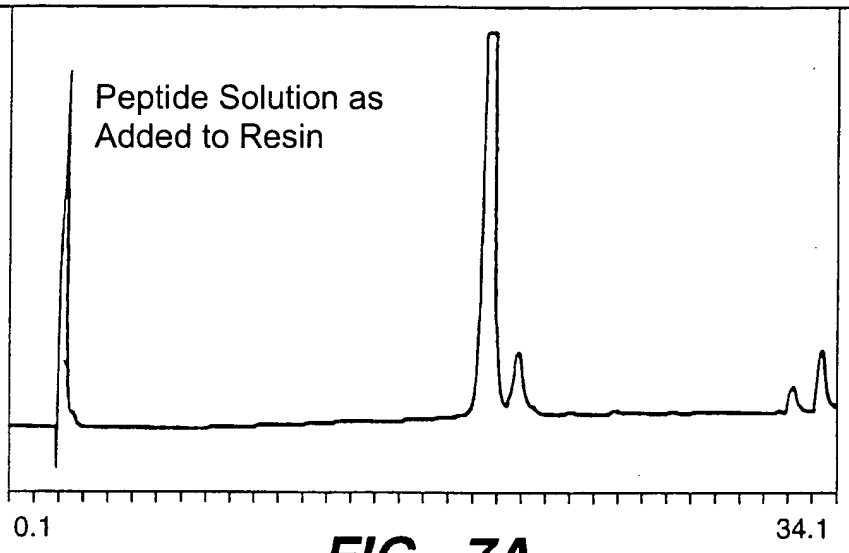


FIG._7A

Polymer-Supported Ligation on PEGA

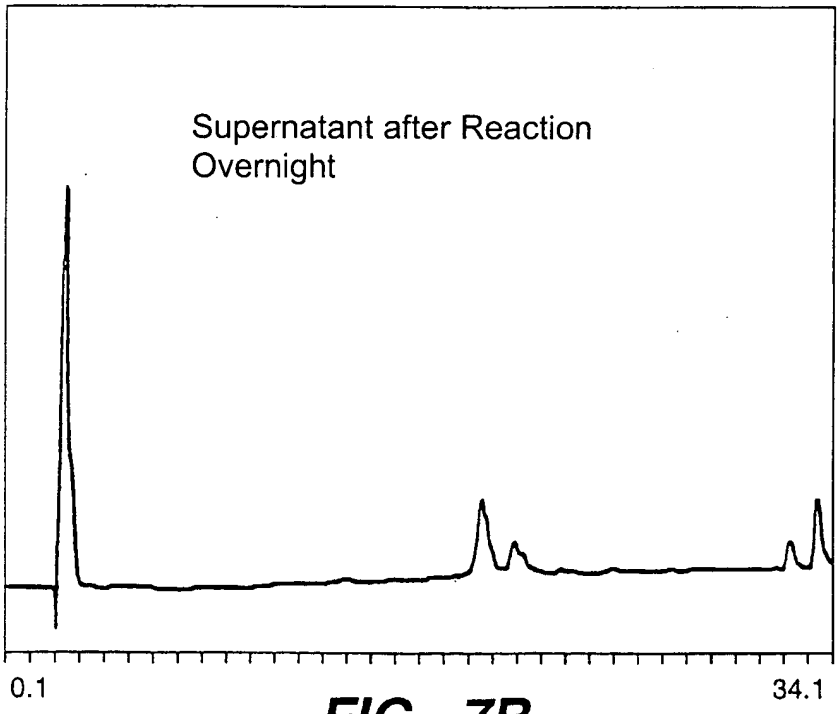


FIG._7B

Lev - *MSC* - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG - *COSH* (1)
+ Resin - PCL - ONH2

↓1. pH 4.6, 6M Gu•HCl, 0.1 ACETATE

Resin - *PCL* - *oxime* - *MSC* - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG - *COSH* (1)

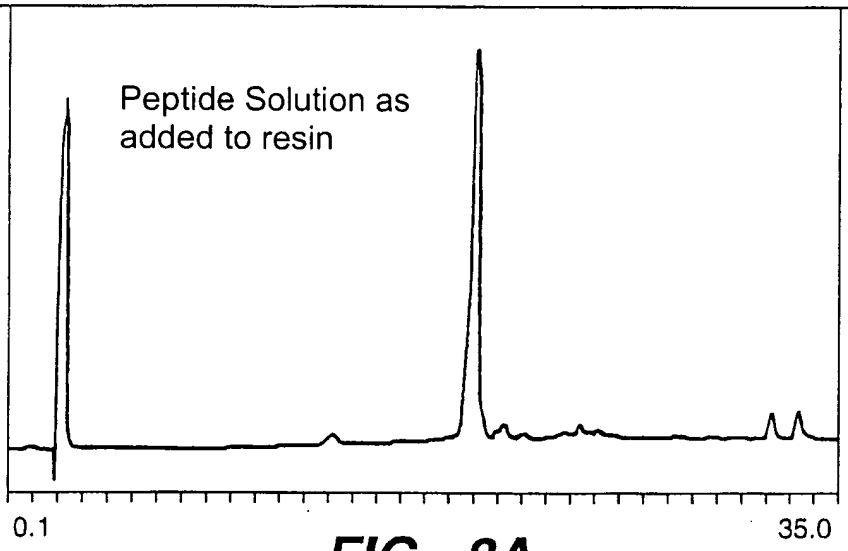


FIG._8A

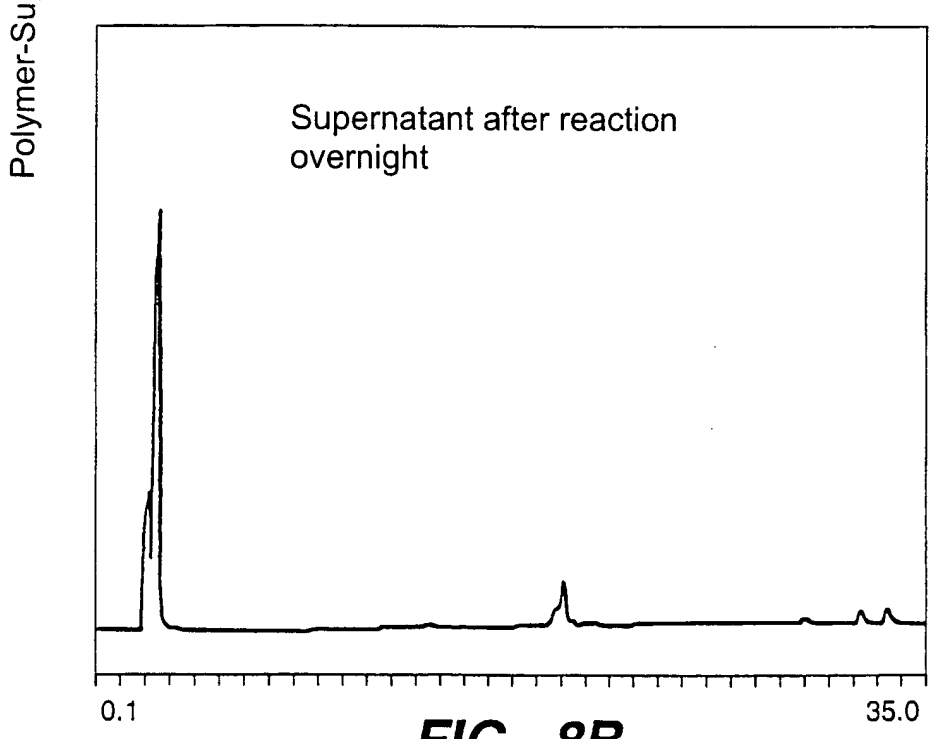


FIG._8B

Lev - *MSC* - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG - *COSH* (1)
+ Resin - PCL - ONH2

↓1. pH 4.6, 6M Gu•HCl, 0.1 ACETATE

Resin - *PCL* - *oxime* - *MSC* - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG - *COSH* (1)
MALDI MASS = 4022, BASE CLEAVAGE MASS = 3745

Polymer-Supported Ligation on ISCO

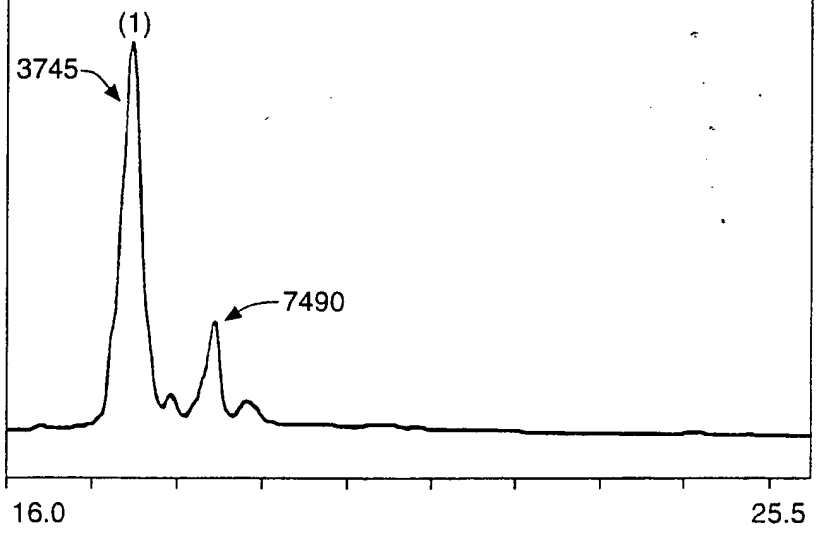


FIG._9A

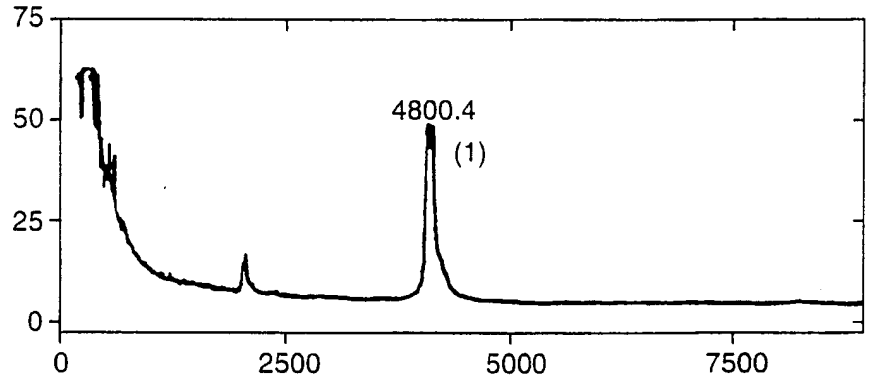


FIG._9B

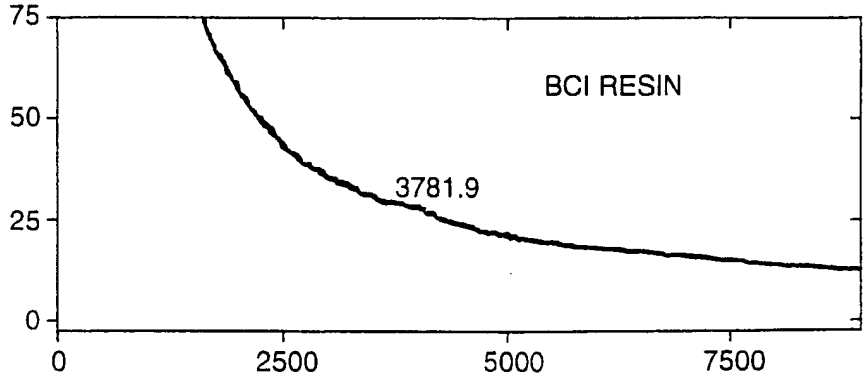


FIG._9C

Resin - PCL - oxime - MSC - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG - *COSAc* (1)
MALDI MASS = 4080, BASE CLEAVAGE MASS = 3729
+ H - CGFRVREFGDNTA - COSH (2)

↓3. pH 7.5, 6M Gu•HCl, 0.1M PHOSPHATE, 0.5% THIOPHENOL

Resin - PCL - oxime - MSC - LTEGLHGFHVHEFGDNTAGCTSAGPHFNPLSRKHG**CGFRVREF - GDNTA** - *COSH* (1+2)
MALDI MASS = 5476, BASE CLEAVAGE MASS = 5199

Polymer-Supported Ligation on ISCO

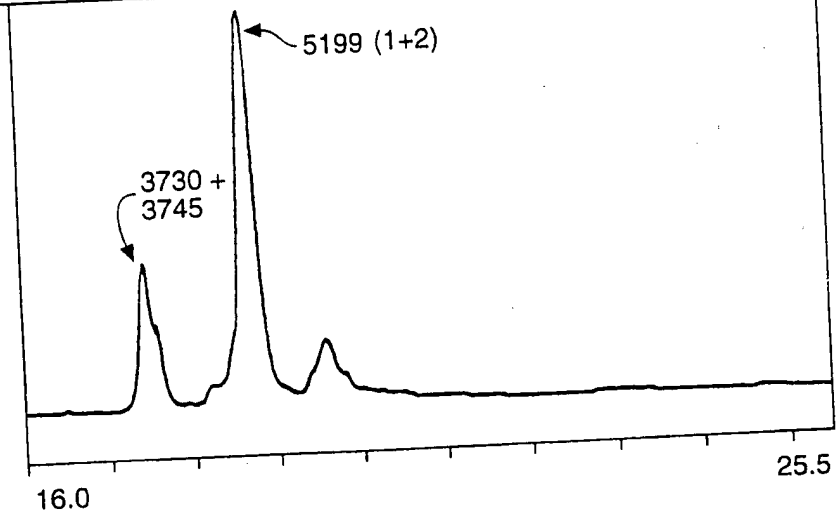


FIG._10A

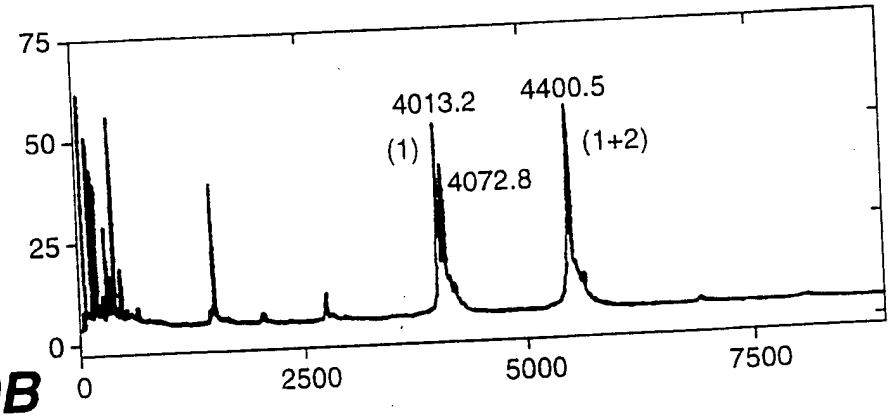


FIG._10B

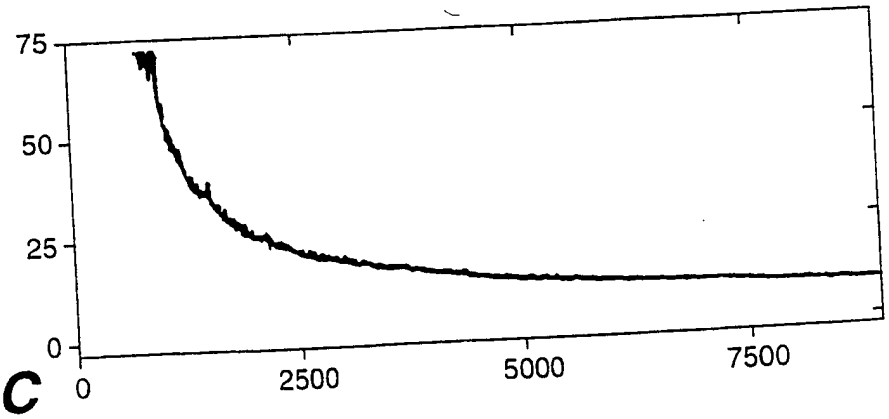


FIG._10C

Polymer-Supported Ligation on ISCO

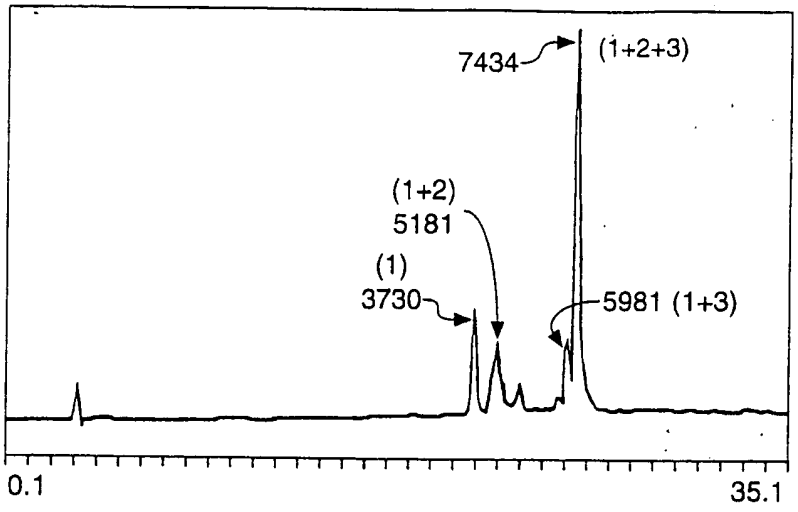


FIG._11

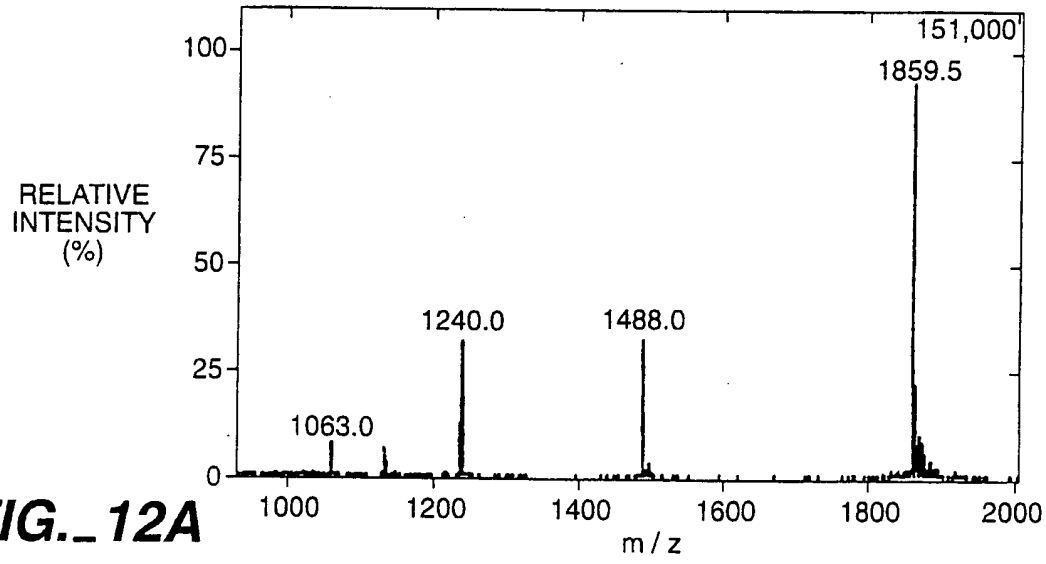


FIG._12A

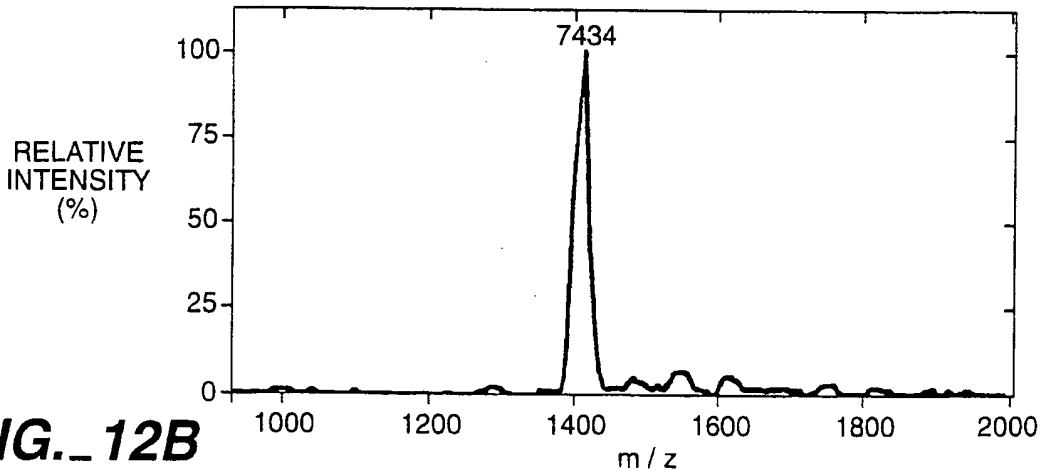


FIG._12B

Polymer-Supported Ligation on PEGA

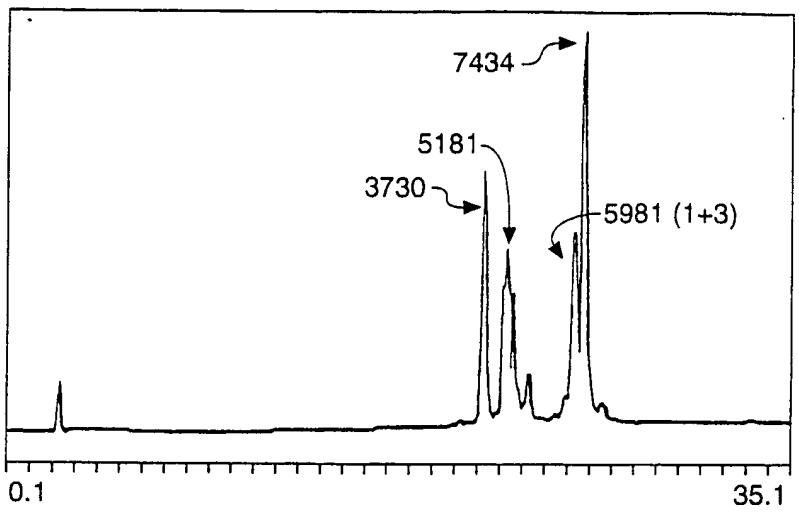


FIG._13

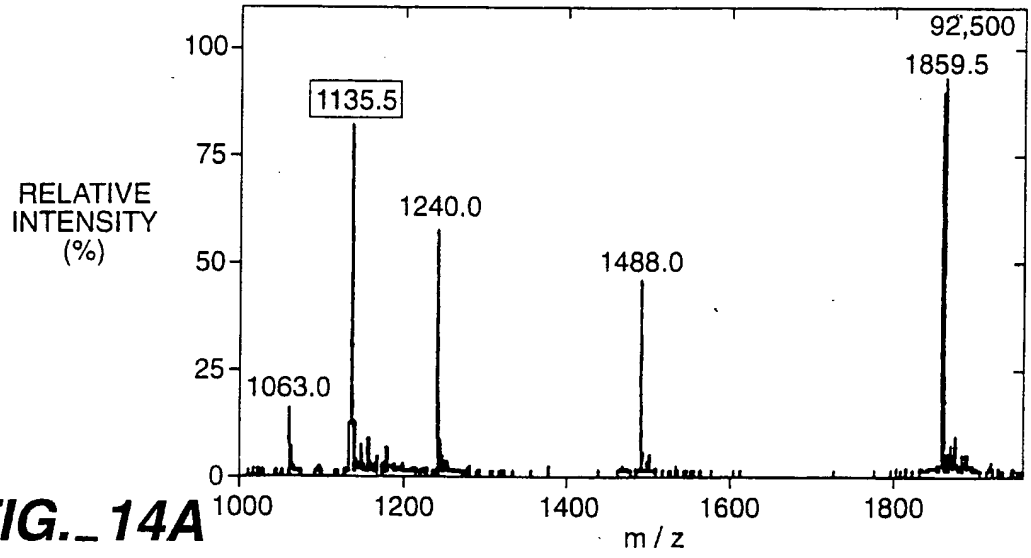


FIG._14A

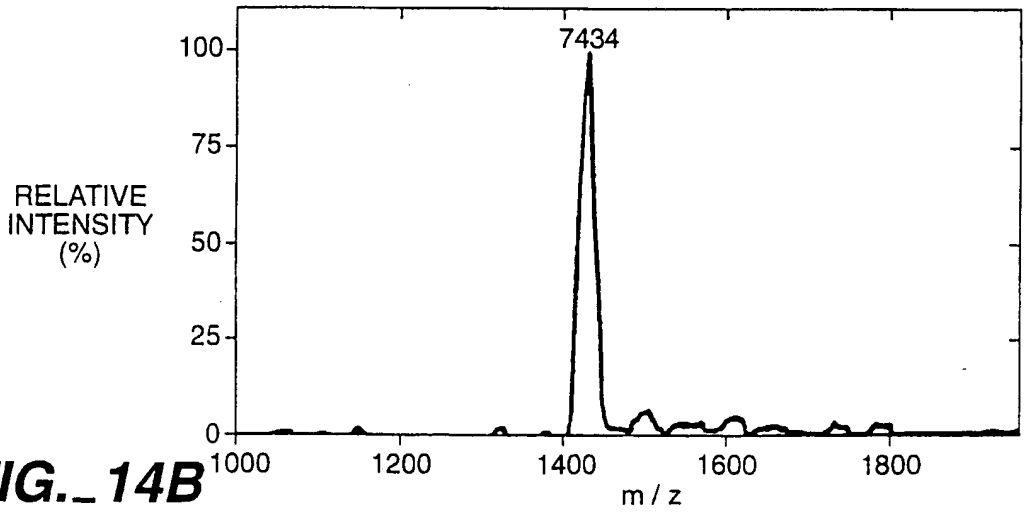


FIG._14B

On Resin
Purification

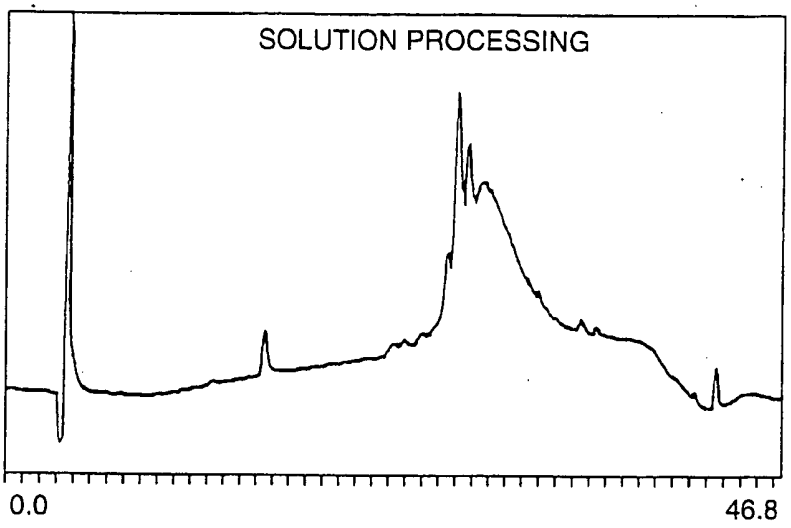


FIG._15A

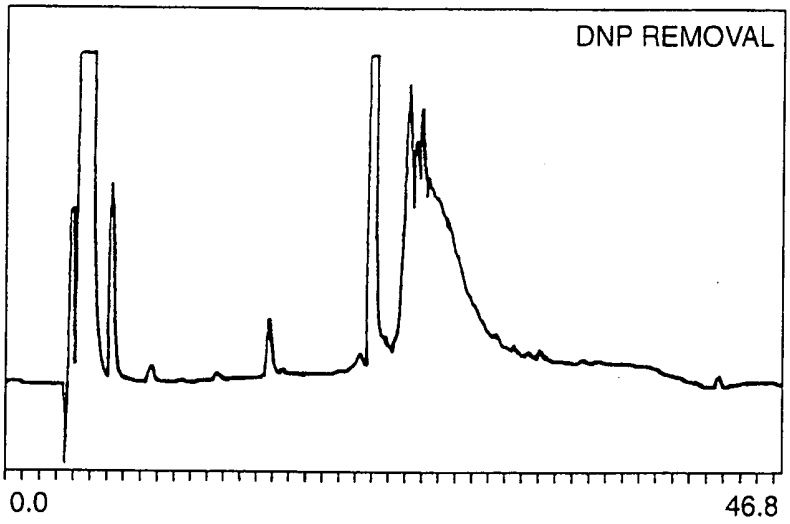


FIG._15B

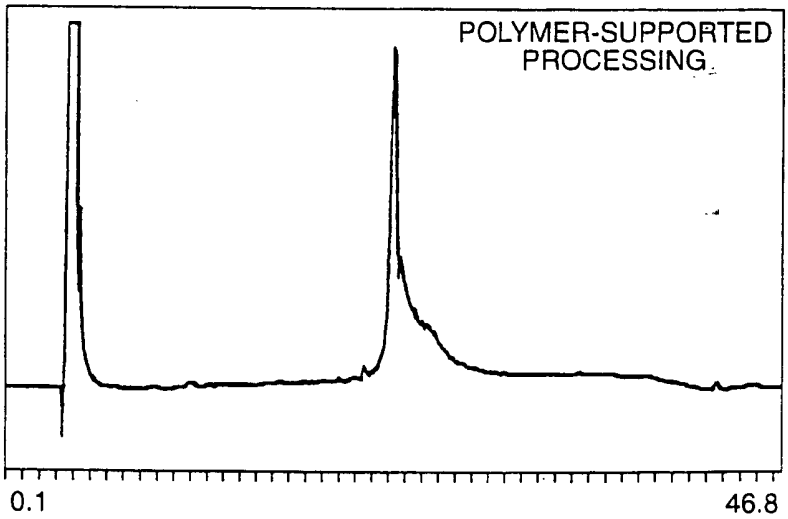


FIG._15C

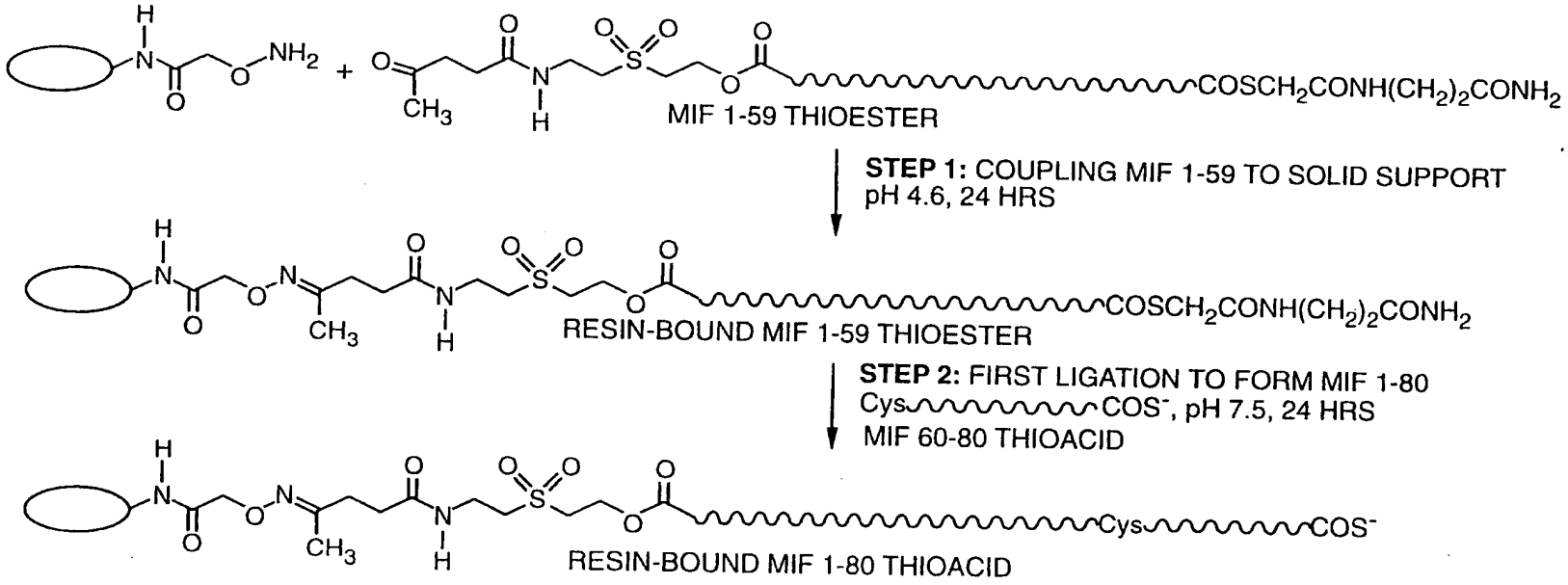


FIG._16A

Synthesis of MIF by Solid Phase Native Ligations

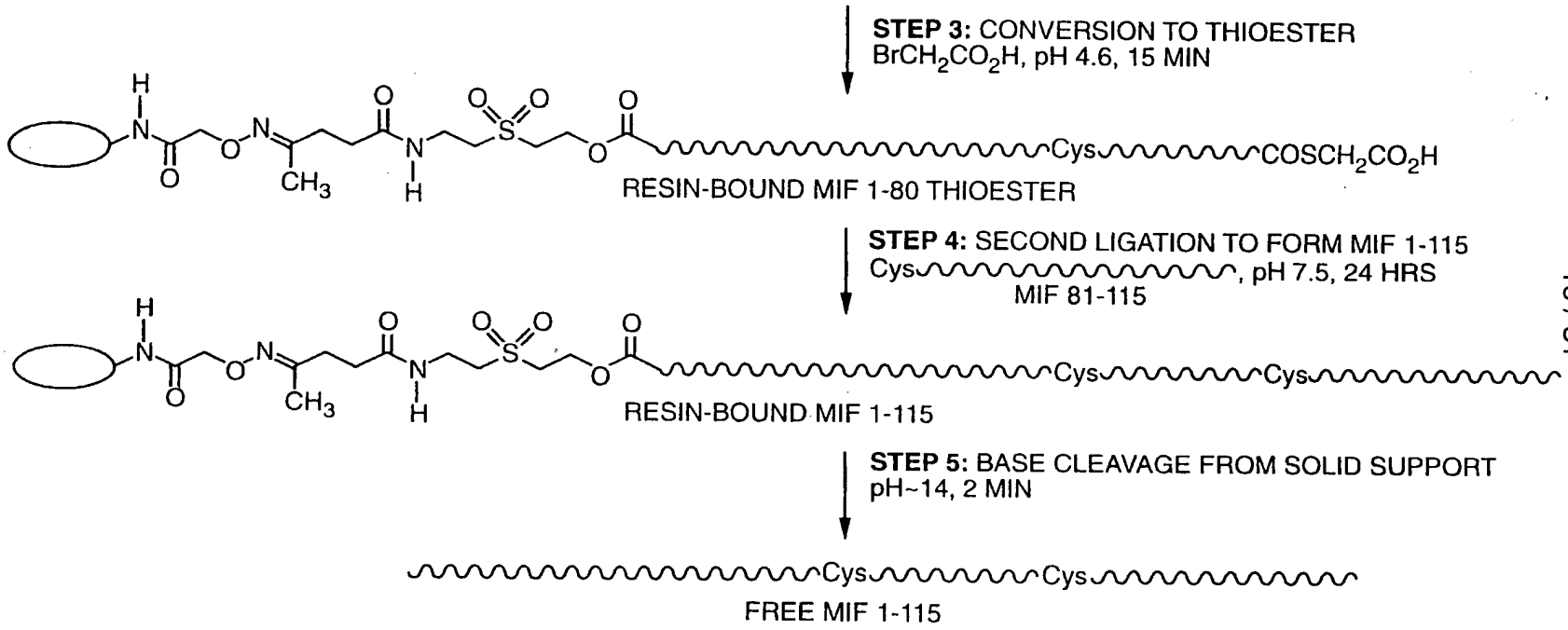

STEP 3: CONVERSION TO THIOESTER
BrCH2CO2H, pH 4.6, 15 MIN
Cys
COSCH2CO2H
RESIN-BOUND MIF 1-80 THIOESTER
STEP 4: SECOND LIGATION TO FORM MIF 1-115
Cys
, pH 7.5, 24 HRS
MIF 81-115
Cys
Cys
RESIN-BOUND MIF 1-115
STEP 5: BASE CLEAVAGE FROM SOLID SUPPORT
pH~14, 2 MIN
Cys
Cys
FREE MIF 1-115

FIG._16B

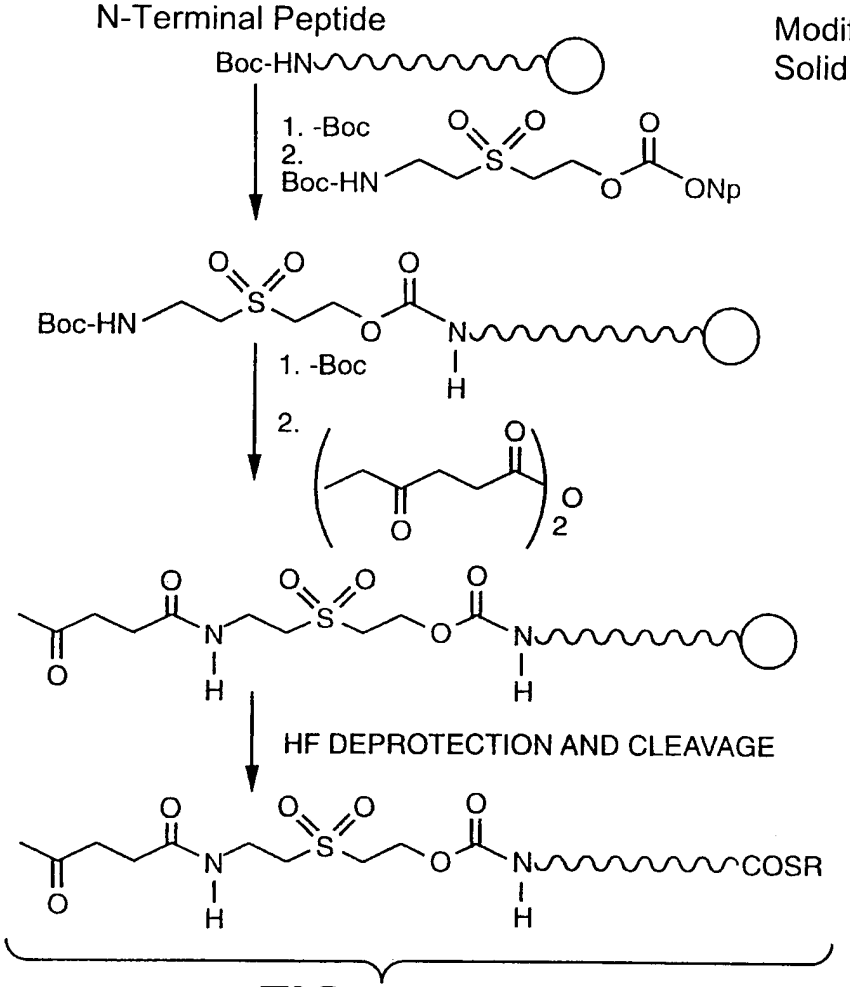


FIG._17A

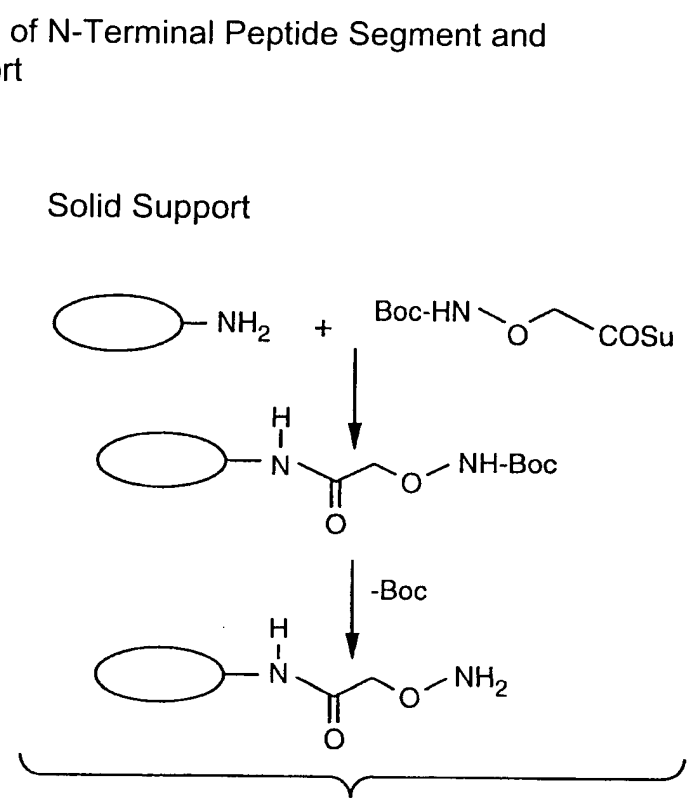
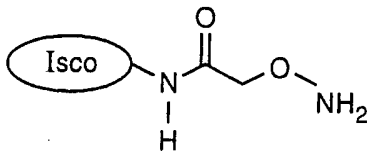


FIG._17B

Coupling of MIF 1-59 to Solid Support



+

KETONE - MSC HANDLE - MET^{1} - MIF 2 - 58 - Leu^{59} - SAc - βAla - CO_2H

#1

↓

Isco — OXIME - MSC HANDLE - MET^{1} - MIF 2 - 58 - Leu^{59} - SAc - βAla - CO_2H

EXPECTED BASE CLEAVAGE MASS = 6271

FIG._18A

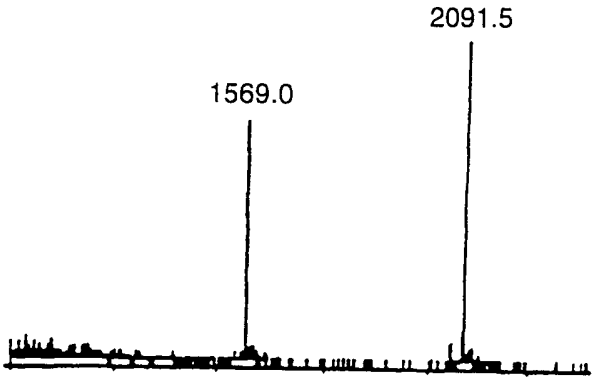


FIG._18C

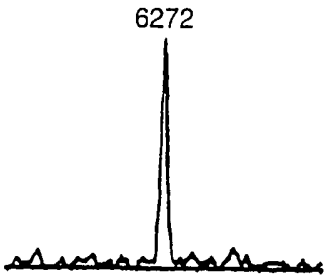
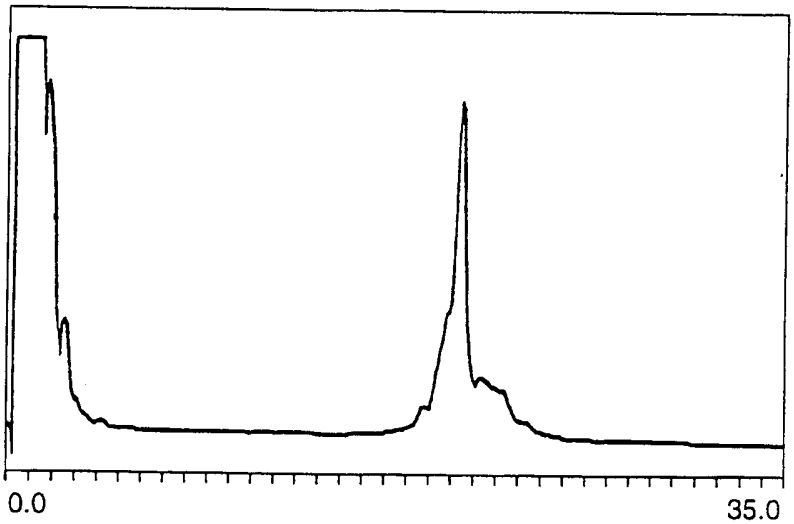


FIG._18D

FIG._18B



Ligation to form MIF 1-80

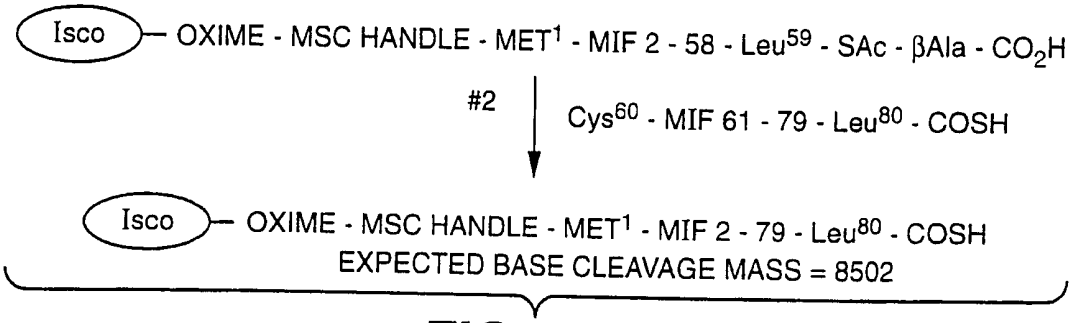


FIG._19A

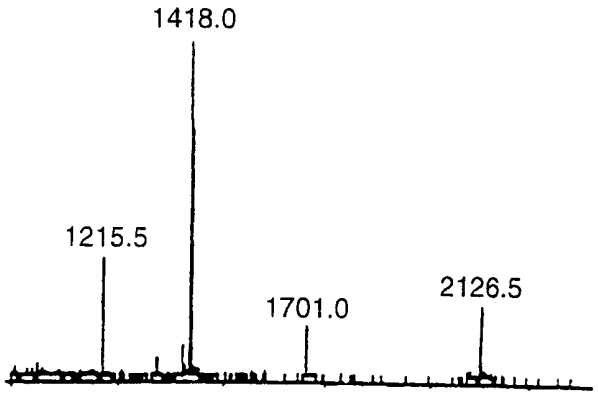


FIG._19C

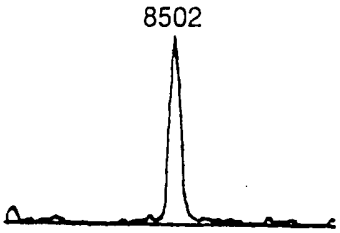


FIG._19D

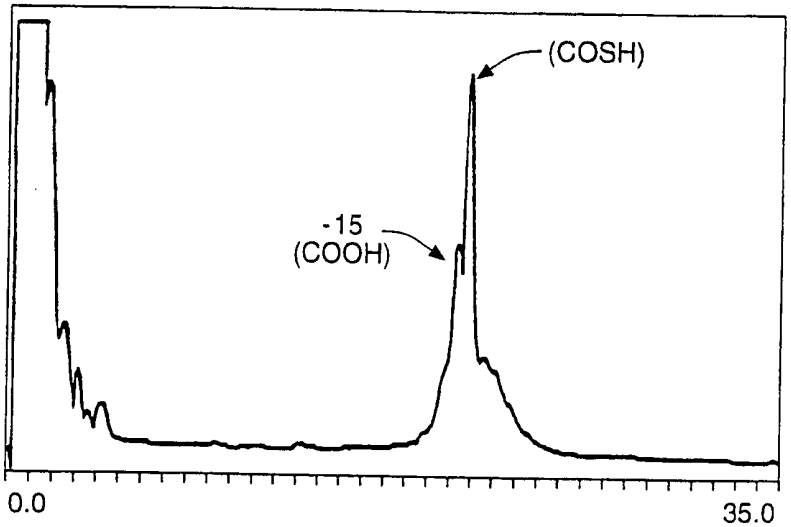


FIG._19B

Ligation to form MIF 1-115

Isco — OXIME - MSC HANDLE - MET^{1} - MIF 2 - 79 - Leu^{80} - COSAc

#4 ↓ Cys^{81} - MIF 82 - 114 - Ala^{115} - CO_2H
6M Gu•HCl, 0.1, 0.1 M Na Pi, 0.5% THIOPHENOL
0.15 M METHIONINE, pH 7.5

Isco — OXIME - MSC HANDLE - MET^{1} - MIF 2 - 114 - Ala^{115} - CO_2H
EXPECTED BASE CLEAVAGE MASS = 12450

FIG._20A

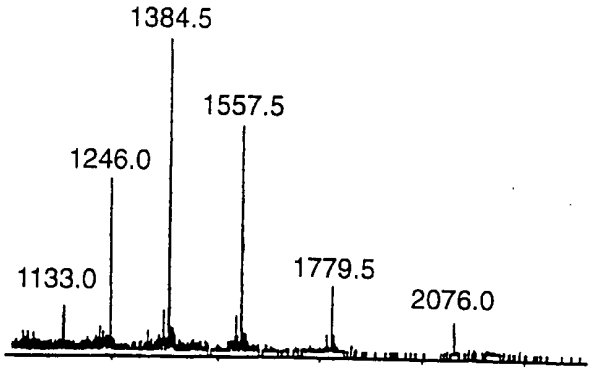


FIG._20C

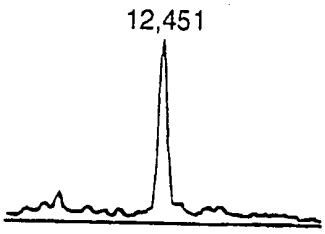


FIG._20D

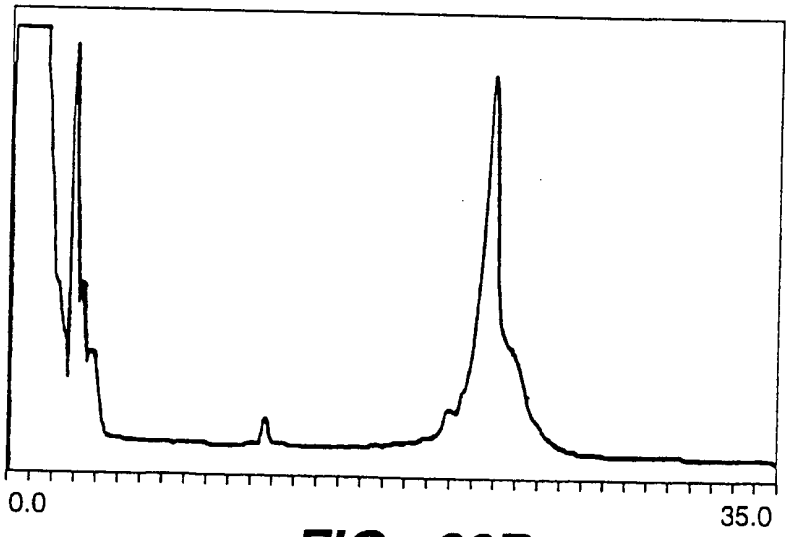


FIG._20B

Solid Phase Chemical Ligations in the
C- to N-terminal Direction

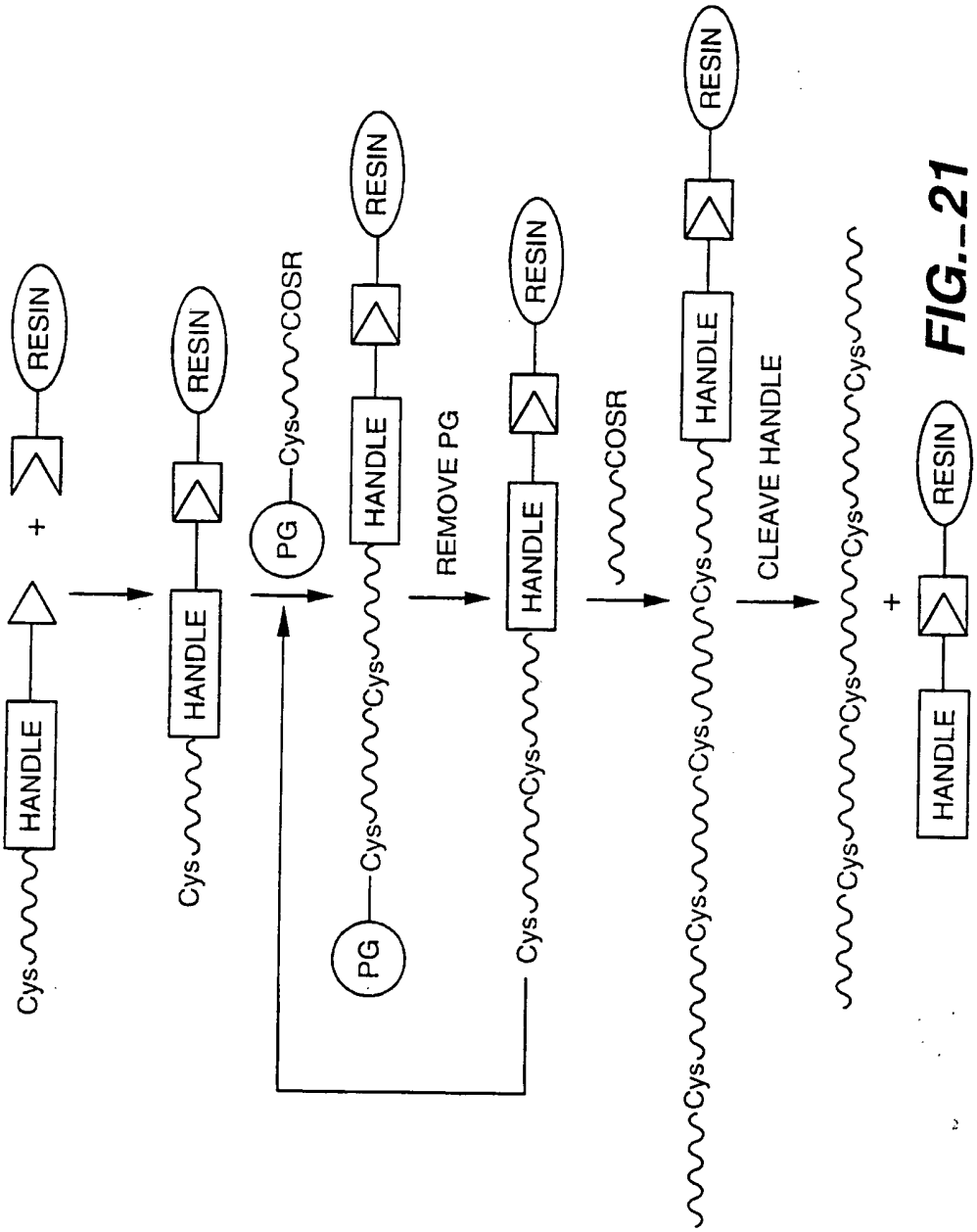
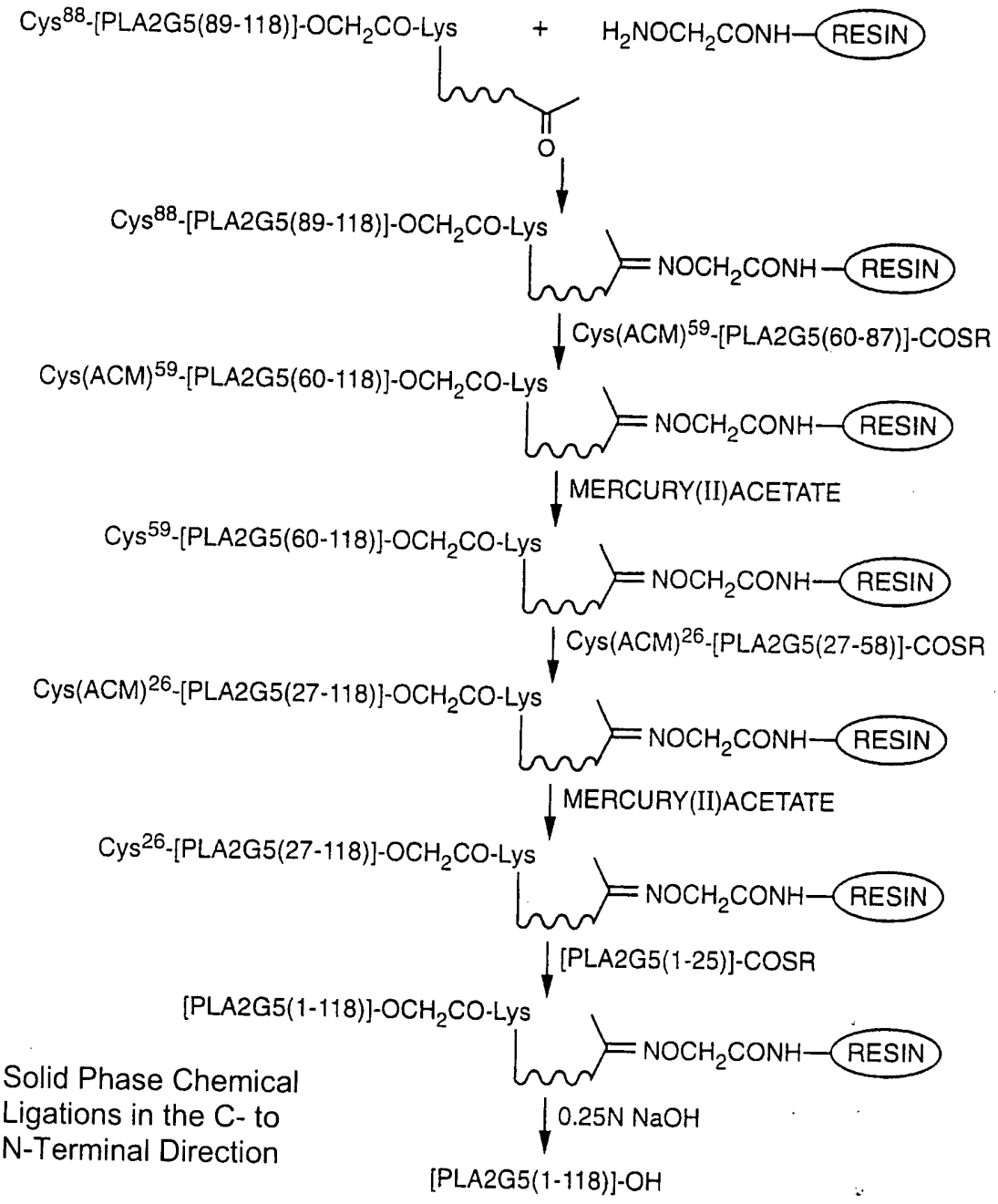


FIG._21



Solid Phase Chemical Ligations in the C- to N-Terminal Direction

Synthesis of Phospholipase A2, Group 5 (PLA2G5)

FIG._22

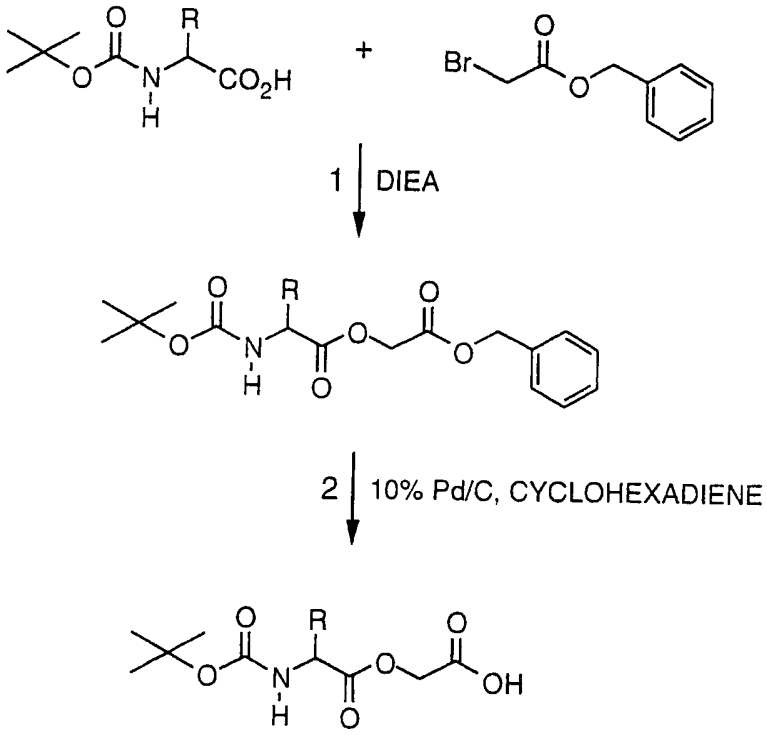
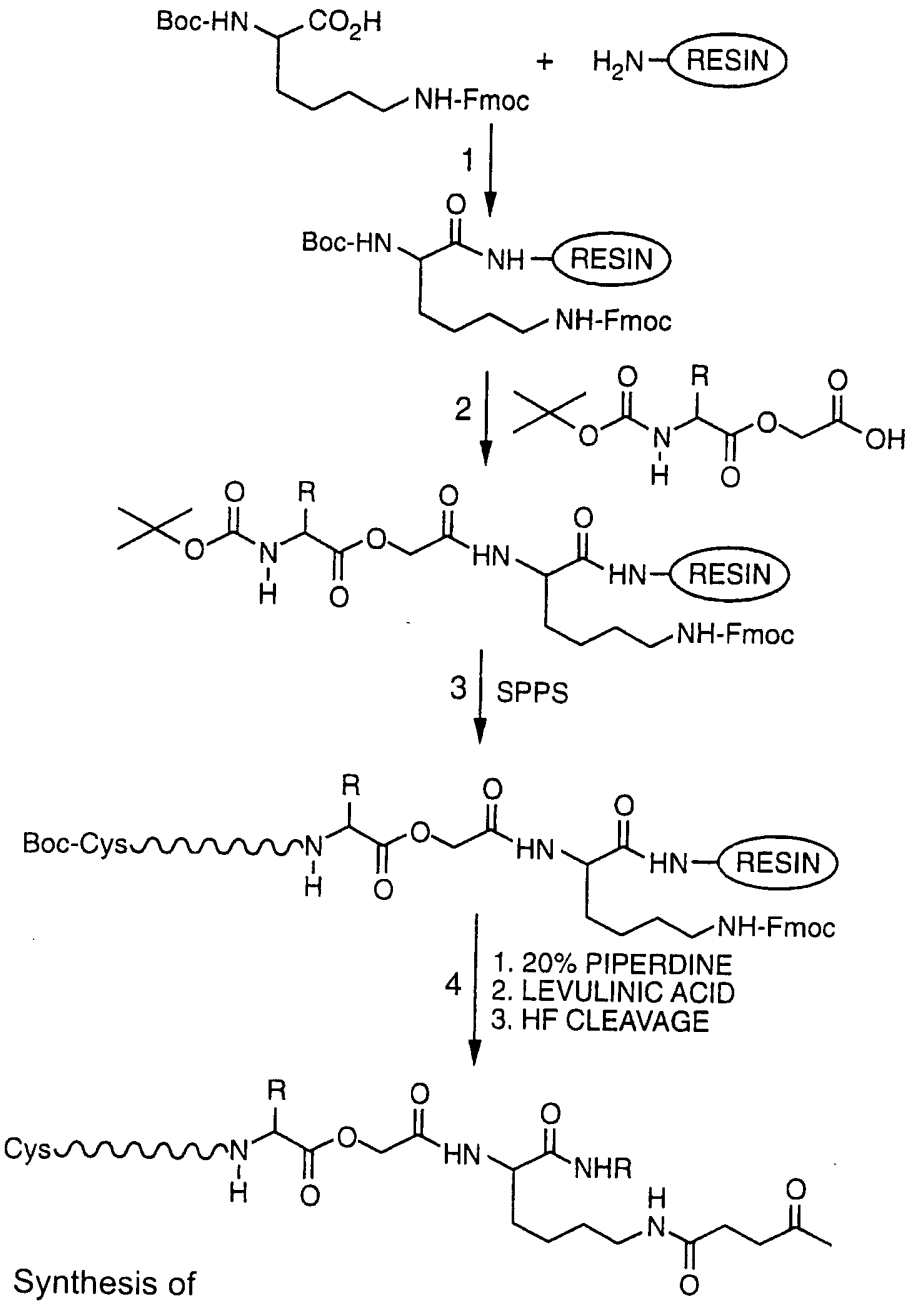


FIG._23

Synthesis of Cam ester derivative



Synthesis of
C-Terminal Peptide
Segment

FIG._24

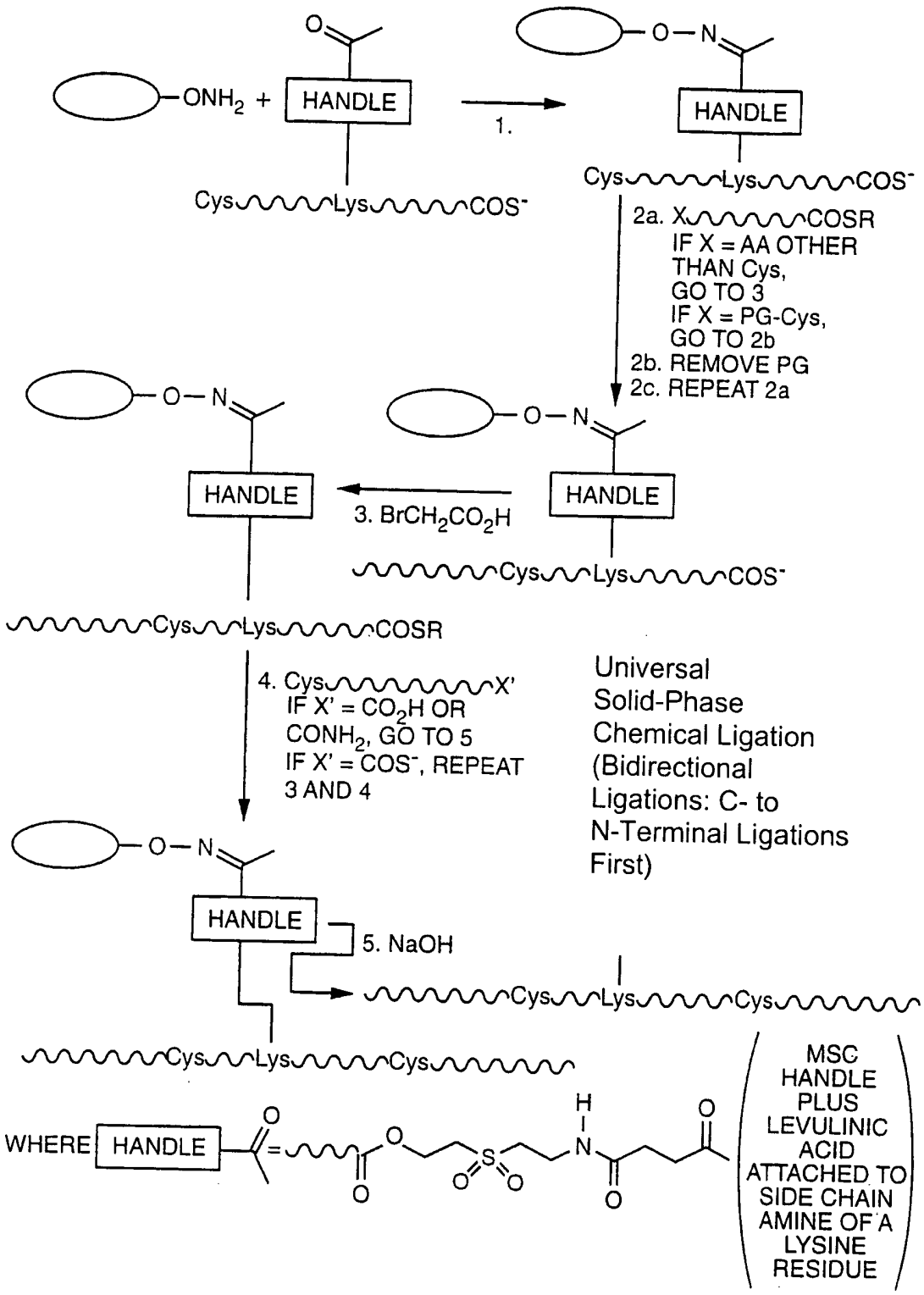

ONH2 +
HANDLE
1.
Cys Lys COS-
2a. X COSR
IF X = AA OTHER THAN Cys, GO TO 3
IF X = PG-Cys, GO TO 2b
2b. REMOVE PG
2c. REPEAT 2a
3. BrCH2CO2H
Cys Lys COSR
4. Cys X'
IF X' = CO2H OR CONH2, GO TO 5
IF X' = COS-, REPEAT 3 AND 4
Universal Solid-Phase Chemical Ligation (Bidirectional Ligations: C- to N-Terminal Ligations First)
5. NaOH
WHERE HANDLE
MSC HANDLE PLUS LEVULINIC ACID ATTACHED TO SIDE CHAIN AMINE OF A LYSINE RESIDUE

FIG._25A

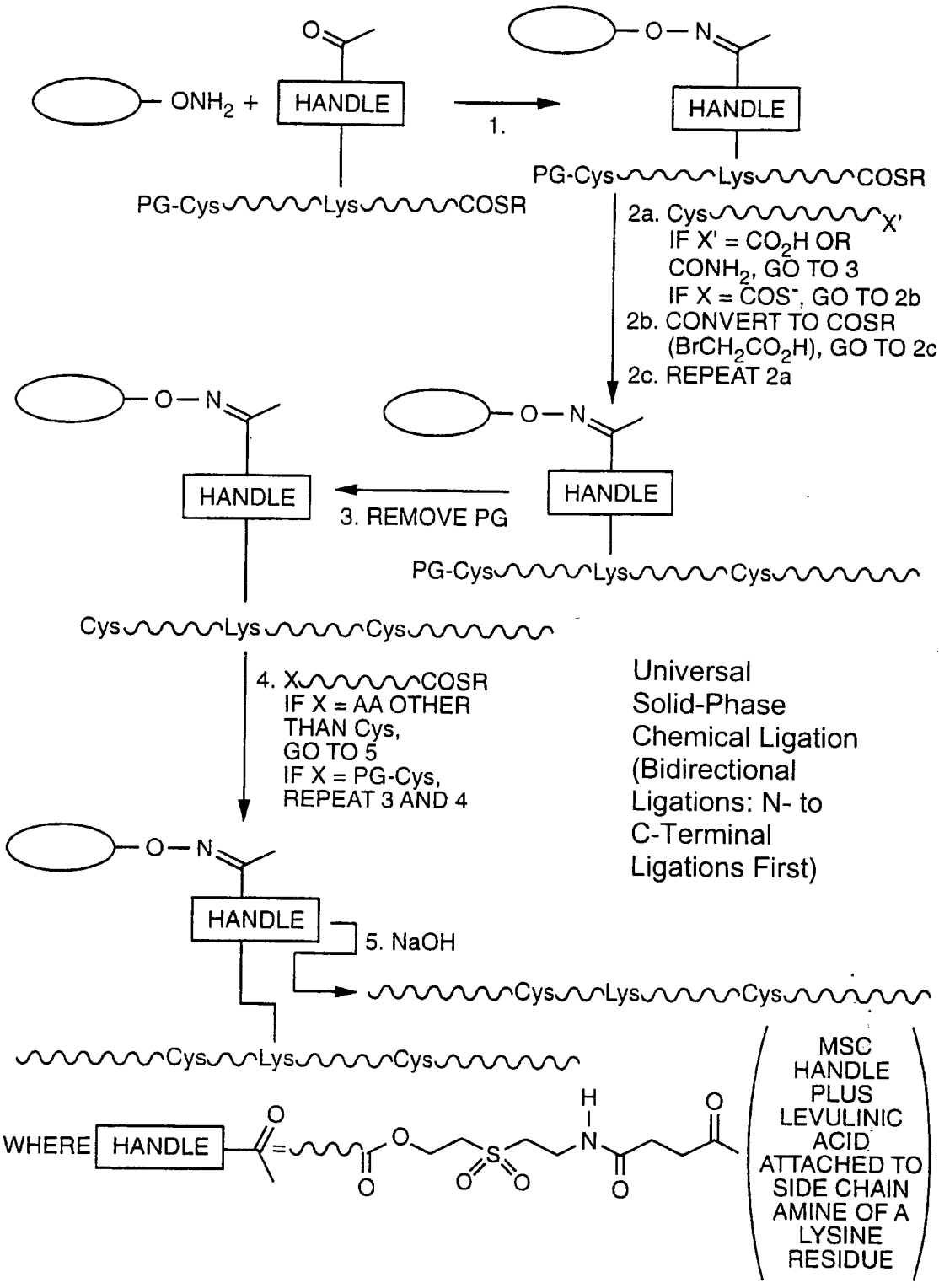

ONH2 +
HANDLE
1.
PG-Cys Lys COSR
2a. Cys X'
IF X' = CO2H OR CONH2, GO TO 3
IF X = COS-, GO TO 2b
2b. CONVERT TO COSR (BrCH2CO2H), GO TO 2c
2c. REPEAT 2a
3. REMOVE PG
Cys Lys Cys
4. X COSR
IF X = AA OTHER THAN Cys, GO TO 5
IF X = PG-Cys, REPEAT 3 AND 4
Universal Solid-Phase Chemical Ligation (Bidirectional Ligations: N- to C-Terminal Ligations First)
5. NaOH
WHERE HANDLE
MSC HANDLE PLUS LEVULINIC ACID ATTACHED TO SIDE CHAIN AMINE OF A LYSINE RESIDUE

FIG._25B

Synthesis of Modified Peptide Segment for Universal Solid Phase Chemical Ligation

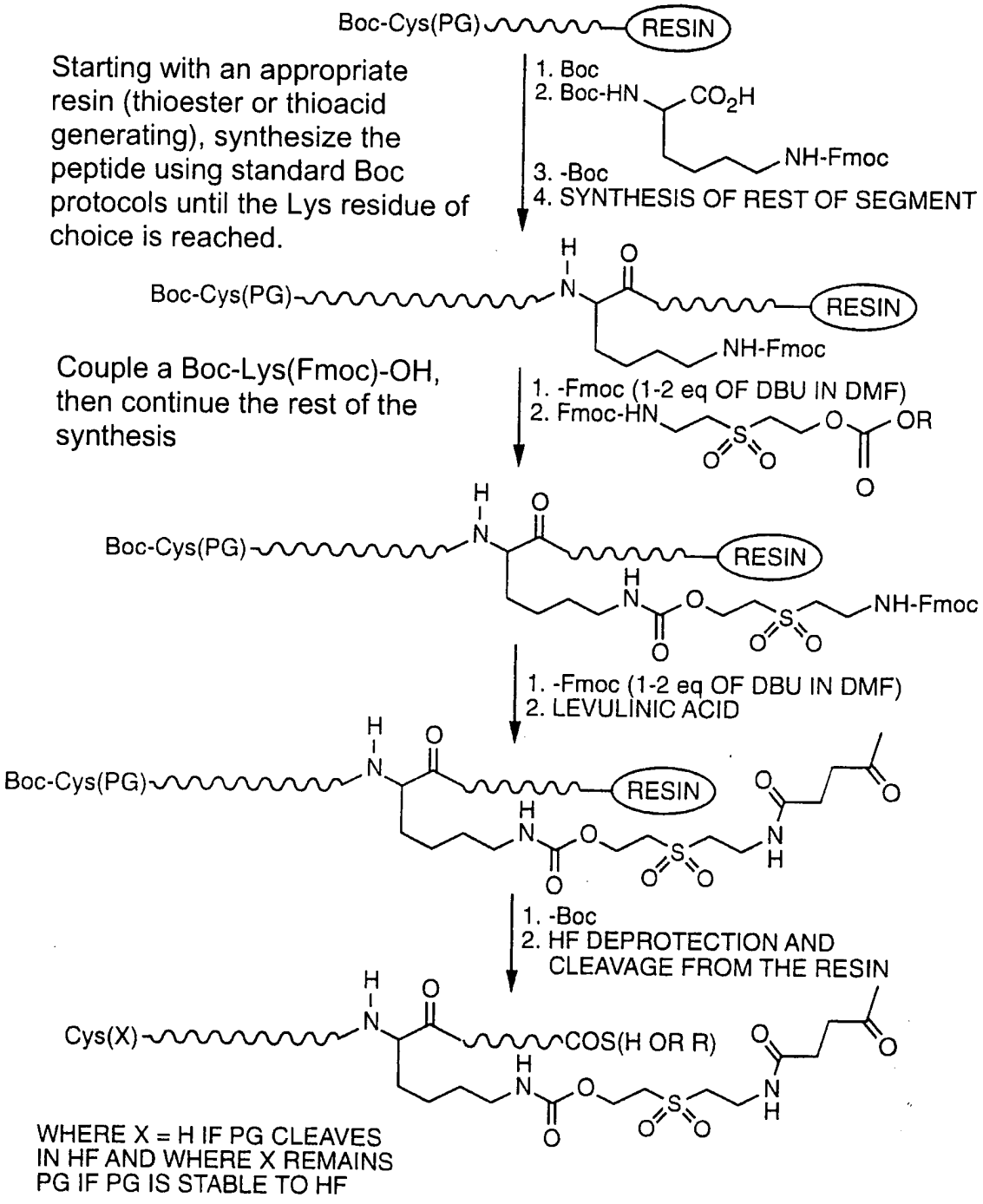


FIG._25C

Synthesis of
C-Terminal
Peptide Segment

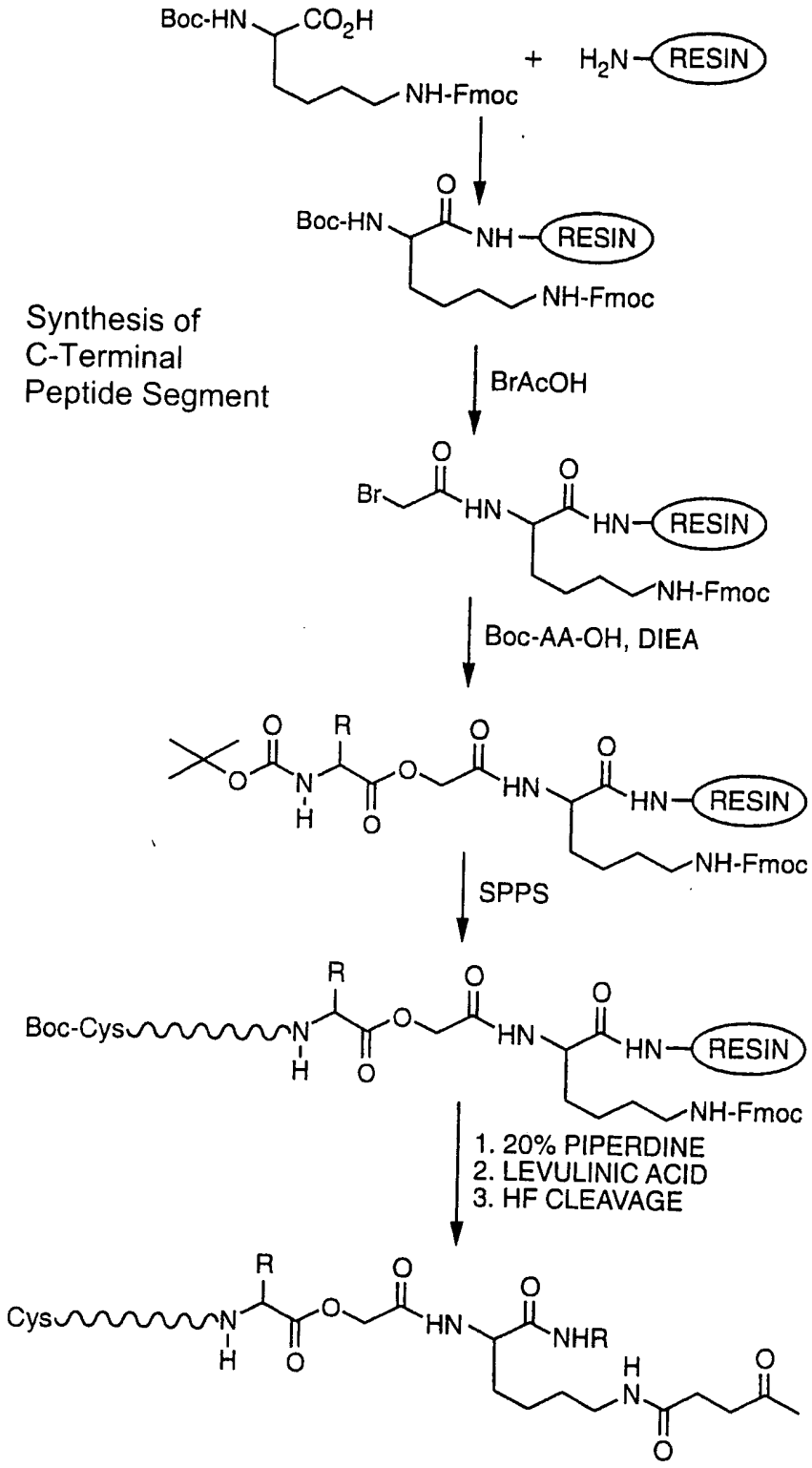


FIG._27

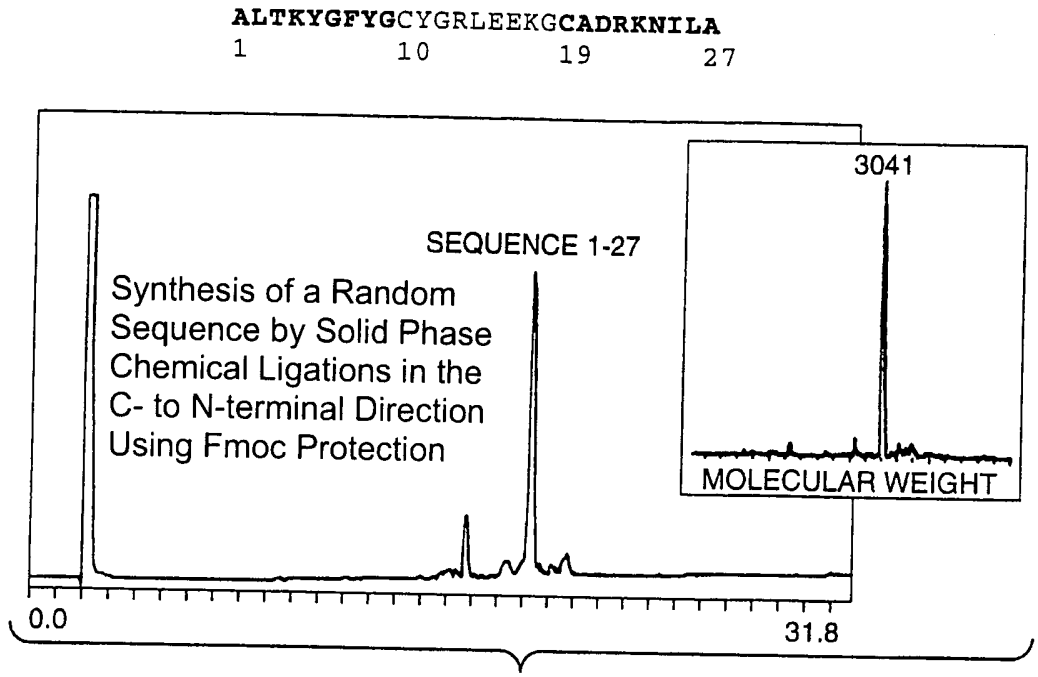

ALTKYGFYGCYGRLEEKGCADRKNILA
1
10
19
27
3041
SEQUENCE 1-27
Synthesis of a Random Sequence by Solid Phase Chemical Ligations in the C- to N-terminal Direction Using Fmoc Protection
MOLECULAR WEIGHT
0.0
31.8

FIG._28

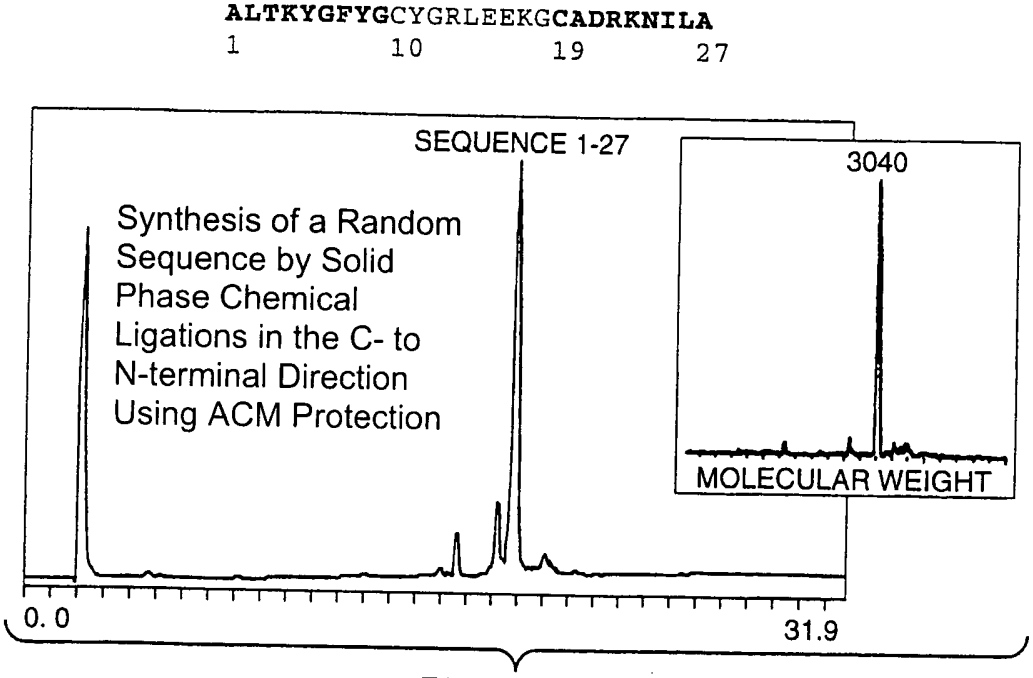
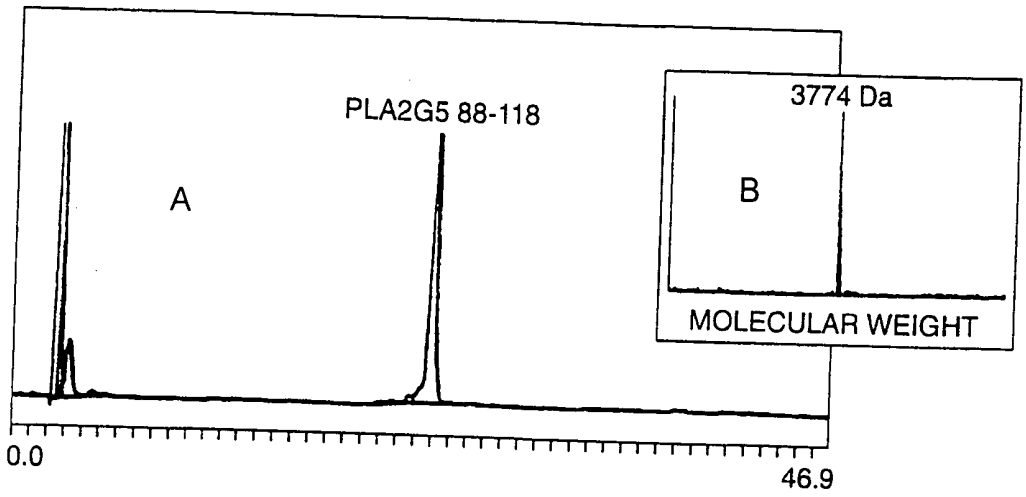

ALTKYGFYGCYGRLEEKGCADRKNILA
1
10
19
27
SEQUENCE 1-27
3040
Synthesis of a Random Sequence by Solid Phase Chemical Ligations in the C- to N-terminal Direction Using ACM Protection
MOLECULAR WEIGHT
0. 0
31.9

FIG._29

1 26 59
GLLDLKSMIEKVTGKNALTNYGFYG**CYCGWGGRGTPKDGTDWCCWAHDHCYGRLEEKGC**
NIRTQSYKYRFAWGVVTCEPGPFCHVNL**CACDRKLVYCLKRNLRSYNPQYQYFPNILCS**
88 118



1 26 59
GLLDLKSMIEKVTGKNALTNYGFYG**CYCGWGGRGTPKDGTDWCCWAHDHCYGRLEEKGC**
NIRTQSYKYRFAWGVVTCEPGPFCHVNL**CACDRKLVYCLKRNLRSYNPQYQYFPNILCS**
88 118

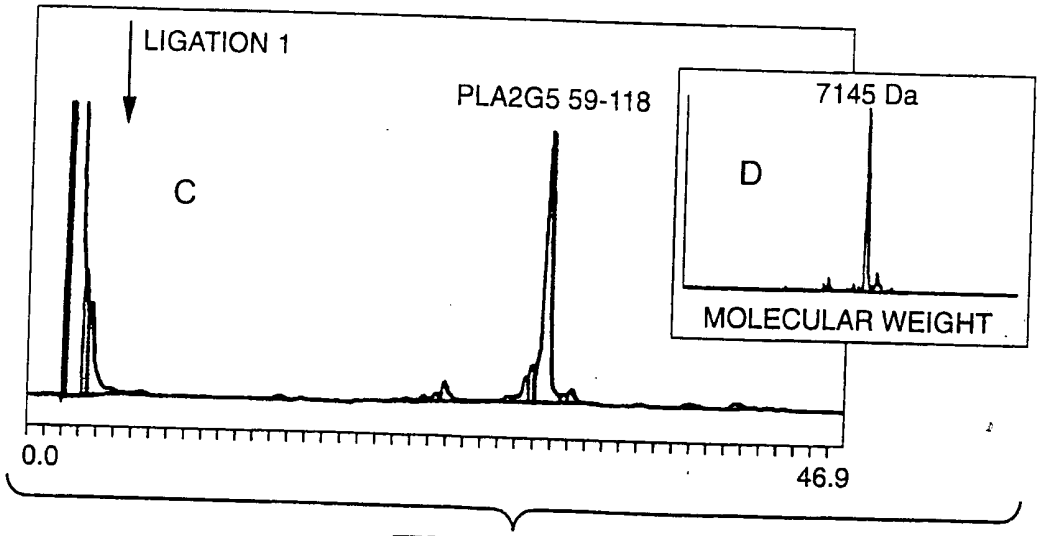
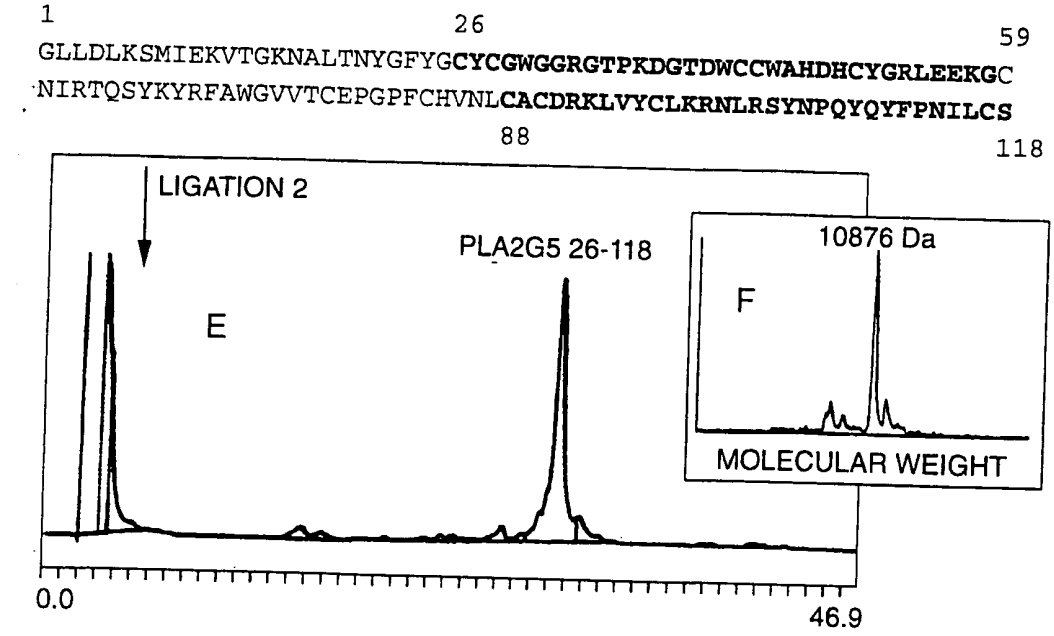


FIG._30


1
26
59
GLLDLKSMIEKVTGKNALTNYGFYGCYCGWGGRGTPKDGTDWCCWAHDHCYGRLEEKGC
NIRTQSYKYRFAWGVVTCEPGPFCHVNLCACDRKLVYCLKRNLRSYNPQYQYFPNILCS
88
118
LIGATION 2
PLA2G5 26-118
E
10876 Da
F
MOLECULAR WEIGHT
0.0
46.9

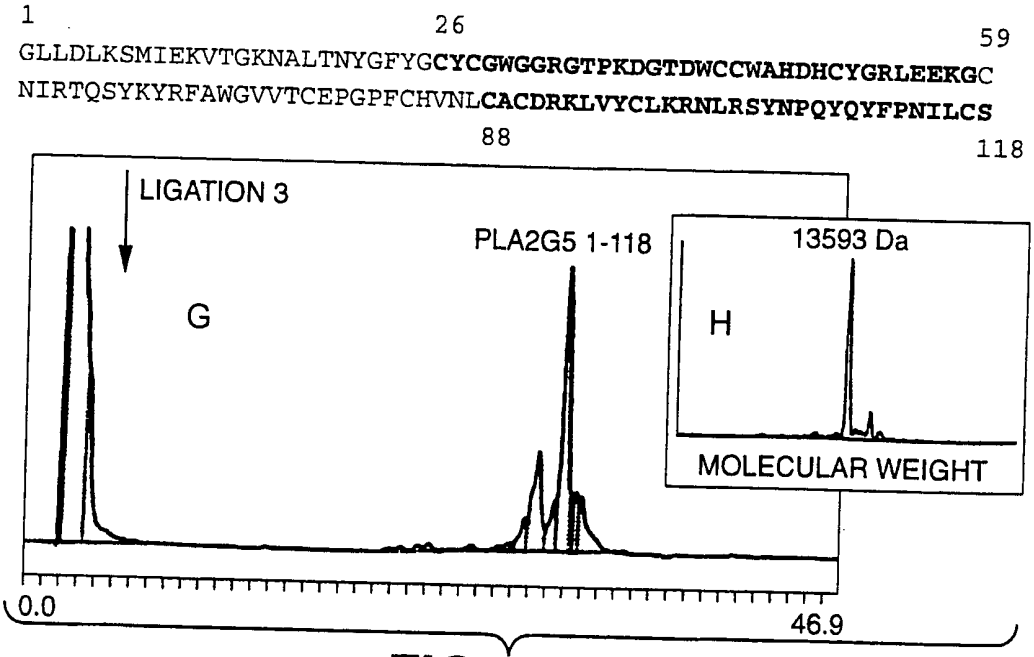

1
26
59
GLLDLKSMIEKVTGKNALTNYGFYGCYCGWGGRGTPKDGTDWCCWAHDHCYGRLEEKGC
NIRTQSYKYRFAWGVVTCEPGPFCHVNLCACDRKLVYCLKRNLRSYNPQYQYFPNILCS
88
118
LIGATION 3
PLA2G5 1-118
G
13593 Da
H
MOLECULAR WEIGHT
0.0
46.9

FIG._30